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## THE ORIGIN OF RESPIRATORY ACTIVITY PATTERNS<sup>1, 2</sup>

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Recent observations upon the activity of the respiratory neurons of the dog, obtained by electrical soundings of the exposed medulla, have established three patterns of discharge (Gesell, Magee and Bricker, 1940): 1, the slowly augmenting type of discharge in which activity builds up slowly and subsides abruptly; 2, the rapidly augmenting type in which activity builds up quickly and subsides slowly; 3, the steady state discharge in which a uniform activity is periodically maintained. The slowly augmenting discharges occurred almost exclusively during the inspiratory phase of breathing while the rapidly augmenting and steady state discharges occurred almost exclusively during the phase of expiration. Histological analysis revealed that all patterns of activity were found at all neuron stations established along the respiratory arcs. It may, therefore, be assumed that the slowly augmenting discharges arise in structures concerned in the conduct of the inspiratory act and that the rapidly augmenting and steady state discharges arise in the structures concerned in the expiratory act. This agrees with the view generally held of the existence of an inspiratory and an expiratory half center but leaves undetermined the factors responsible for the characteristic configuration of the respiratory discharges. The solution of this problem may well supply the key to the integration of the respiratory act.

Most important for the moment is the need of knowing whether such patterns of potentials as have been found by direct observation (Gesell, Magee and Bricker, 1940) are basically of central or reflexogenic origin. So far as we know that point has not received direct study. Attention has been focussed on the question of whether or not the centers possess

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<sup>2</sup> The authors wish to express their thanks to Merek and Company for supplying the curare which was used.

automaticity, or whether or not the periodic showers of proprioceptive signals set up with each respiratory act are essential for the periodic repetition of that act. The fact that breathing will begin spontaneously after prolonged acapnic pauses is evidence that inspiration can be initiated and maintained by continuous respiratory drives. It is improbable that periodic proprioceptive signals would continue to circulate during apnea and start a new series of respiratory acts when chemical stimulation reaches threshold value. Furthermore the long continued rhythmic discharges of the respiratory center such as was demonstrated by the action potentials of the phrenic nerve during complete curare paralysis by Winterstein (1911) and Bronk and Ferguson (1935) offer sure confirmation that periodic stimulation is unnecessary to breathing. Yet none of these observations bear on the more pressing problem of the underlying forces determining the *configuration of the activity patterns*.

It is not illogical to assume that the slowly augmenting pattern of the inspiratory discharge could be produced by a progressively increasing proprioceptive drive initiated at the opening of the inspiratory contraction. Indeed such reinforcement has been more recently demonstrated (Worzniak and Gesell, 1939). For example, when tension in the inspiratory muscle develops with the onset of contraction its proprioceptive endings would be distorted and send a correspondingly increased number of signals to the centers. Granting for the moment that such signals are excitatory to the motor cells of their respective muscles, the intensity of contraction would automatically increase. This in turn would produce more muscle tension and greater reflexogenic drive leading to a progressive increase in muscular contraction such as is commonly recorded during normal breathing. On the other hand the possibility of a purely central mechanism of progressive augmentation of contraction cannot be neglected. Such a central basic mechanism would offer a substructure upon which the secondary reflexogenic mechanism could build.

A study of the configuration of the respiratory patterns of discharge during complete motor paralysis produced by curare should offer a clue to the solution of this problem. Since periodic potentials of mechanical or proprioceptive origin are automatically eliminated when artificial ventilation is discontinued, and since there is no reason to suspect the existence of other slowly augmenting groups of sensory potentials we assume that all sensory signals advancing on the centers would be of the steady state pattern (barring of course a gradual increase in the chemoreceptive signals with progressive asphyxia). In other words the centers would be functioning upon a mass of steady state signals of varied origin, arising from the chemical and physical state of its sensory endings. If under the conditions of such drive a periodic slowly augmenting discharge emanates along the phrenic nerve the characteristics of that discharge

should be logically attributable to some phenomenon occurring within the centers.

The records of the inspiratory action potentials of the phrenic nerve published by Winterstein and by Bronk and Ferguson during curare paralysis unfortunately are not clear on this point. We have, therefore, studied the activity of the phrenic nerve in the totally paralyzed and completely anaesthetized dog, kept alive by artificial ventilation. Records were obtained under two conditions: 1. During short periods in which ventilation was completely suspended. 2. During prolonged pe-

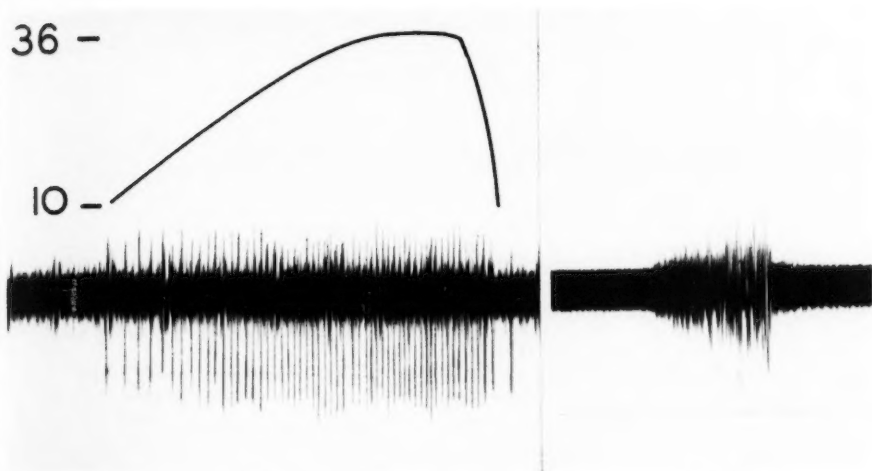


Fig. 1

Fig. 2

Fig. 1. A characteristic slowly augmenting inspiratory discharge of a single motor fibre of the phrenic nerve of the dog. This record was obtained during complete motor paralysis produced by an intravenous injection of curare. The vagus nerves are sectioned. Frequency of firing is plotted on the ordinates above the original electrogram.

Fig. 2. A characteristic slowly augmenting inspiratory fusillade discharge of the phrenic nerve trunk during complete motor paralysis.

riods of artificial ventilation in which the time relation of pulmonary inflation and respiratory discharges were continually changing.

**RESULTS.** *The inspiratory discharge pattern.* The inspiratory discharges were followed by recording the efferent potentials of the phrenic nerve. From figures 1 and 2 it will be seen that the characteristic configuration of inspiratory activity continued after induction of complete motor paralysis. Figure 1 shows the changes in frequency of discharge of a single motor fiber, duplicating the progressively increasing frequency during the inspiratory phase and the sudden falling off of the discharge at the

onset of expiration common to normal breathing. The triangular configuration of the frequency curve is indistinguishable from those already published for reticular cells, ventral horn cells, motor nerve fibers and muscle (Gesell, Magee and Bricker, 1940). Figure 2, a fusillade discharge obtained from the phrenic nerve trunk, after complete motor paralysis, is also indistinguishable from comparable fusillades obtained before the administration of curare. Such records, as was pointed out before (Gesell, Magee and Bricker, 1940) present a picture of the summation of coincident signals of that nerve. The rising level of the potentials is, therefore, dependent upon an increasing coincidence of potentials from a progressively increasing frequency of discharge of the individual fibers and from a progressive recruitment of newly activated units. The persistence of the triangular fusillade is, therefore, significant.

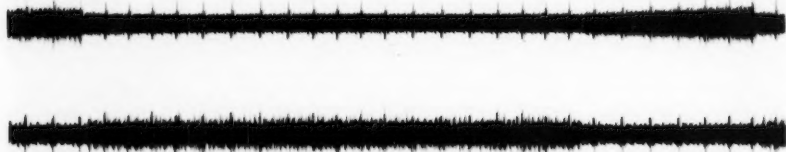


Fig. 3. A comparison of a slowly augmenting inspiratory fusillade of the phrenic nerve trunk (above) and a steady state expiratory fusillade of a nerve branch leading to the transverse abdominal muscle (below). The evenly spaced deflections of low frequency are of cardiac origin.

When artificial ventilation was stopped completely over relatively long periods of time the intensity of the inspiratory fusillades increased as asphyxia progressed, but the configuration maintained its fundamental characteristics. On the other hand when ventilation was held slightly below respiratory requirements a long series of discharges of equal intensity and of constant configuration were obtained. The lack of gross changes in the discharges with changing time relations of pulmonary inflation indicated a weakness of reflex modification of breathing in our experiments. This is attributed to the high level of anesthesia required for the protection of the animals against pain and its consequent diminution of reflexes. It is, therefore, pertinent to note that the phenomena of acceleration and recruitment persist during a complete motor paralysis plus a gross sensory paralysis. Such findings we believe establish a centrifugenic mechanism for both phenomena.

*The expiratory discharge patterns.* The expiratory patterns are more puzzling for under conditions of light anesthesia there may be either the rapidly augmenting or the steady state discharge. Assuming that the

steady state discharge is the basic central pattern, the rapidly augmenting type could well be interpreted as the proprioceptive modification of the basic discharge. To inquire into this possibility we used the same procedure described for the study of the inspiratory discharges, recording in this instance the action potentials of the motor nerves of the abdominal expiratory muscles. Our findings (eleven experiments in all) however showed only the steady state type of discharge before and after curare paralysis. If our concept is correct that the rapidly augmenting type is a reflex modification of the steady state discharge, the absence of this modification before curare paralysis is logically attributable to the gross sensory paralysis obtaining in our experiments. Supporting evidence of the reflex nature of the rapidly augmenting discharge is found in the excitatory action of the vagal stretch reflex which in the case of inspiration builds progressively upon the slowly augmenting central inspiratory discharge. At the end of inspiration vagal signals are at their maximum. If it is true, as preliminary experiments suggest, that the vagal drive can be switched from the inspiratory to the expiratory half center when inspiration gives way to expiration we see a possible explanation of the decreasing force of expiration. Vagal drive is strongest at the beginning of expiration while the lungs are still inflated and weakens as the lungs deflate. The proprioceptive signals would accordingly give decreasing support to the rectangular discharge and thereby change it to the rapidly augmenting type. The persistence of the rhythmic steady state discharges during complete motor paralysis and gross sensory paralysis in turn is evidence that the recurrence of these discharges is independent of rhythmic afferent showers and, therefore, must be centrogenic in origin.

#### SUMMARY AND CONCLUSIONS

A fundamental question of motor integration of the respiratory act is raised: Are the configurations of the activity patterns of the respiratory contractions of centrogenic origin or are they of reflexogenic origin depending upon periodic proprioceptive drives accompanying each phase of the respiratory act?

The answer is sought in a study of activity patterns before and after elimination of periodic proprioceptive signals by complete curare paralysis.

The existence of the characteristic slowly augmenting inspiratory discharge in the phrenic nerve during complete curare paralysis leads to the conclusion that this type of discharge is of centrogenic origin for it occurs without the aid of periodic proprioceptive impulses.

Conclusions regarding the expiratory discharges are more involved for there are two patterns to consider.

The persistence of only the steady state discharge in the motor nerves of the expiratory muscles during curare paralysis indicates that the re-

currence of this discharge is of centrogenic origin for it likewise occurs without the aid of proprioceptive impulses.

In the light of these and other experiments, the rapidly augmenting type of expiratory discharge, found in the absence of motor paralysis and during lighter anesthesia, is tentatively interpreted as a reflex modification of the steady state discharge.

#### REFERENCES

- BRONK, D. W. AND L. K. FERGUSON. This Journal **110**: 700, 1935.  
GESELL, R. Science, in press.  
GESELL, R., C. S. MAGEE AND J. W. BRICKER. This Journal, **128**: 615, 1940.  
WINTERSTEIN, H. Pflüger's Arch. **138**: 159, 1911.  
WORZNIAK, J. J. AND R. GESELL. This Journal **126**: P658, 1939.

## ELECTROLYTE AND WATER EXCHANGES BETWEEN MAMMALIAN MUSCLE AND BLOOD IN RELATION TO ACTIVITY<sup>1</sup>

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Numerous investigators have studied the changes in electrolyte composition and water content of voluntary muscle following contraction.

The most extensive investigations have been carried out by Fenn and his collaborators, who determined changes resulting from 30 minute periods of stimulation by analyzing paired muscles, one of the pair being analyzed at rest to serve as a control for the stimulated muscle. Fenn (1936) in his review on this subject observed the following results of stimulation: 1, a loss of potassium from the cells in exchange for sodium; 2, an increase in the interstitial space resulting from the addition of an ultrafiltrate of the plasma; 3, an additional uptake of water by the cells; 4, a more or less complete reversal of these changes during two hours of recovery.

The methods used to determine these changes in nearly all the investigations have the disadvantage that only relatively large exchanges can be detected. Long periods of stimulation are necessary to produce changes of this magnitude.

We can find no direct observations in the literature as to whether such electrolyte and water exchanges do take place as a result of relatively short periods of contraction such as might occur normally in the intact animal.

It is obvious that such changes as would occur from short periods of contraction could not be detected by direct muscle analyses. We have attempted to determine these changes by carrying out analyses of arterial and venous blood supplying the muscle in question. In order to carry out such an investigation with any degree of success it is necessary to have a set up in which: 1, the composition of the arterial blood supplying the muscle is very nearly constant; 2, the blood flow through the muscle can

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be determined accurately throughout the experiment; 3, arterial and venous blood samples can be taken easily without disturbing any of the conditions affecting the muscle in question.

It is difficult to meet these requirements using an intact animal. Therefore we have used a heart lung gastrocnemius preparation, since the heart lung is one of the more nearly physiological perfusion systems available.

**METHODS.** The process of isolating the gastrocnemius of the dog with blood and nerve supply intact has previously been described by Banus (1936). The femoral artery and vein were dissected out carefully from the inguinal flexure to their points of entry into the gastrocnemius muscle. All branches were double ligated and cut between the ligatures. Care was taken so that the circulation to the gastrocnemius was interfered with as little as possible during the dissection. The sciatic nerve was dissected out from the upper thigh to the gastrocnemius. After these procedures were completed, the gastrocnemius was exposed and the tendon severed distally. The lower leg was then detached at the knee joint leaving the gastrocnemius attached to the distal end of the femur. A steel drill was then inserted into the femur just proximal to the condyles to serve as a means of subsequently clamping the femur in place.

The femur was then transected proximal to the drill and hemorrhage from the cut end arrested by means of bone wax. During this time a heart lung preparation was made; the heart and lungs were removed from the chest and placed in a constant temperature bath as described by Peters and Visscher (1936). A "Y" tube was placed in the tubing carrying the arterial blood from the brachio-cephalic artery to the artificial resistance, so that part of the arterial output of the heart lung could be diverted to perfuse the gastrocnemius. The arterial resistance was constant throughout the experiment so that the mean pressure was about 110 mm. of mercury.

When the heart lung preparation was completed, the sciatic nerve supplying the gastrocnemius was transected as far centrally as possible. The femoral artery and vein were double ligated and cut between the ligatures as far centrally as the dissection permitted. The gastrocnemius preparation consisting of: gastrocnemius muscle, distal end of the femur, sciatic nerve, femoral artery and vein was weighed on a spring balance; the artery and vein immediately cannulated; and the circulation restored by arterial defibrinated whole blood from the heart lung. A period of 2 to 5 minutes elapsed between the tying off of the vessels to the muscle and the resumption of the circulation through the muscle by means of the heart lung preparation.

The femur was then clamped in place upon a steel rod and the tendon secured by means of a cord to a clamp on the lower end of the rod so that the gastrocnemius was suspended along the rod under a small tension.

The sciatic nerve was drawn through a Sherrington electrode.

The cannulae, supporting rod, and electrode all passed through rubber stoppers in a brass plate which was clamped upon a large glass cylinder after the muscle was in place. The brass plate and cylinder were constructed so as to be water tight. The completed preparation was then immersed in the constant temperature bath with the heart lung. The interior of the glass cylinder was kept at atmospheric pressure by means of a rubber tube communicating with the room air.

The venous cannula drained directly into a graduated cylinder through a short piece of rubber tubing. The venous pressure was therefore approximately zero. Blood flow was determined by timing the venous outflow into graduated cylinders with a stop watch.

When the preparation was completed and in the bath, glucose was added to the venous reservoir sufficient to make an addition of 100 mgm. for every 100 cc. of blood. At the start of the experiment the blood volume was about 1.5 liters. The large reserve volume in the reservoir made it possible to study exchanges for periods up to 30 minutes without adding more blood or returning blood perfused through the muscle to the reservoir. The venous reservoir was equipped with a mechanical stirrer to insure as nearly as possible a constant composition of the blood perfusing the gastrocnemius. Morphine and ether were used as an anesthetic for the dog from which the gastrocnemius was isolated. The heart lung dog was anesthetized with *nembutal*.

Blood flows were taken for a period of about 30 minutes to allow time for equilibration of the system and thorough mixing of the blood. Resting arterial and venous blood samples were then taken. The arterial sample was taken slowly from a side tube in the arterial tubing leading to the muscle, and the venous sample collected directly from the venous outflow. The muscle was then stimulated via the sciatic nerve by means of a Harvard inductorium. A tetanic current was used in most of the experiments, intermittent or steady for periods of from  $1\frac{1}{2}$  to  $4\frac{1}{2}$  minutes. Venous and arterial samples and blood flows were taken throughout the stimulation period and for varying lengths of time afterward.

At the end of the experiment the preparation was again weighed so that a rough determination of the change in weight of the muscle during the perfusion period could be made.

Part of each blood sample was reserved for whole blood analyses. The remainder was centrifuged in 15 cc. recalibrated conical centrifuge tubes at 3000 R.P.M. for 30 minutes in order to determine cell volume. These determinations were run in duplicate, 10 cc. portions of blood being used for each determination. The serum was withdrawn soon after centrifugation and reserved for analyses.

Blood and serum water and specific gravities were determined by weighing either 1 or 2 cc. (pipetted) portions of blood before and after drying in an oven at 95 to 100 degrees centigrade for 48 hours. Potassium content of whole blood and serum was determined by a modification of the silver cobalti-nitrite method of Breh and Gaebler (1930). Chloride was determined by the method of Van Slyke and Sendroy (1923). Duplicate analyses were carried out on all chemical determinations.

**RESULTS.** A series of 12 experiments on 12 different preparations has been carried out. Due to the large number of chemical determinations on each experiment and the numerous calculations based upon them it is impossible to publish the complete data of all experiments. Therefore the results of each experiment are summarized graphically. Complete tabular data and equations are presented for one experiment.

Since the significance of calculated exchanges based upon arteriovenous differences is determined chiefly by the accuracy of the analyses on which the arterial venous differences are based, the probable errors of the various determinations are given in table 1.<sup>2</sup>

*Experiment G-6 (8/12/38).* The preparation was made as described above. The gastrocnemius had been perfused by the heart lung for one hour before the start of

<sup>2</sup> This table was computed with the assistance of Dr. Alan Treloar from the series of chemical analyses run in these experiments.

stimulation. The muscle was stimulated indirectly as described for a period of 4' 31" with a steady tetanic current. Stimulation was interrupted momentarily several times to promote blood flow through the muscle. A schedule of the samples taken, the venous blood flows, and the analytical determinations is given in table 2.

*Calculations of water exchanges between blood and muscle.* Calculated water exchanges are based on the equation:

$$(1) \quad (100 (H_2O)_A - (H_2O)_X) \div (100 - (H_2O)_X) = (H_2O)_V$$

(H<sub>2</sub>O)<sub>X</sub> = grams water gained by muscle per 100  
grams "incident blood"<sup>3</sup>

(H<sub>2</sub>O)<sub>A</sub> = grams water in 1 gram of arterial blood  
(H<sub>2</sub>O)<sub>V</sub> = grams water in 1 gram of venous blood

TABLE 1  
*Probable error of analyses*

|   | H <sub>2</sub> O |        | POTASSIUM |        | SP. GRAVITY |         | CELL<br>VOLUME,<br>BLOOD |
|---|------------------|--------|-----------|--------|-------------|---------|--------------------------|
|   | Blood            | Plasma | Blood     | Plasma | Blood       | Plasma  |                          |
| Number of duplicate analyses.....                 | 86               | 45     | 125       | 126    | 98          | 56      | 65                       |
| Average value of analyses.....                    | 81.20*           | 93.50* | 21.0†     | 17.0†  | 1.057       | 1.023   | 44.0¶                    |
| Probable error of a single analysis....           | 0.018‡           | 0.018‡ | 0.223§    | 0.227§ | 0.0009‡     | 0.0013‡ | 0.335                    |
| Per cent probable error of a single analysis..... | 0.022            | 0.019  | 1.06      | 1.33   | 0.085       | 0.101   | 0.760                    |

\* Grams per 100 grams.

† Mgm. per 100 grams.

‡ Grams.

§ Mgm.

¶ Volumes per cent.

|| Ce.

This equation is based upon the assumption that the change in dry weight of 100 grams of incident blood is negligible during the passage through the muscle. This assumption is valid if there is no appreciable loss of protein or other large organic molecules from the blood, since the change in dry weight due to exchange of the electrolytes determined in these experiments is small.

The quantity of water exchanged during any period can be calculated by multiplying the value (H<sub>2</sub>O)<sub>X</sub> by the arterial blood flow in terms of 100 grams. The average rate of exchange was then calculated by dividing the quantity exchanged by the time elapsed in seconds.

<sup>3</sup> The term "incident blood" will be used in this paper to replace the more cumbersome term "blood entering the muscle."

Calculations show that if protein did diffuse into the muscle the values for water exchange as calculated above would be too small.

These values as calculated for experiment G-6 are given in table 3.

*Serum and erythrocyte water exchanges.* Serum water exchanges can also be calculated by means of equation (1) given above. In this case

TABLE 2  
*Samples, blood flows and analytical determinations G-6*

|  | REST               |       | STIMULATION |                 |      |      |      |      | RECOVERY     |              |       |                |
|--|--------------------|-------|-------------|-----------------|------|------|------|------|--------------|--------------|-------|----------------|
| Samples*                                   | 1                  | 2     | (3          | 4               | 5    | 6)   | 7    | (8   | 9            | 10)          | 11    | 12             |
| Ce. volume venous sample                   | 40                 | 5     | 5           | 5               | 25   | 5    | 5    | 5    | 5            | 30           | 30    | 51             |
| Duration of period (sec.)                  | 410                | 80    | 26          | 22              | 118  | 25   | 21   | 17   | 16           | 92           | 122   | 360            |
| Rate blood flow (cc. per min.)             | 5.9                | 3.8   | 11.5        | 13.6            | 12.7 | 12.0 | 14.3 | 17.7 | 18.8         | 19.6         | 14.8  | 10.2           |
| Whole blood analyses                       |                    |       |             |                 |      |      |      |      |              |              |       |                |
| H <sub>2</sub> O (grams per cent)          | A 81.18<br>V 81.32 | 81.30 |             | 81.18<br>80.04† |      |      |      |      | 80.88†       | 81.21        | 81.09 | 81.20<br>81.13 |
| K <sup>+</sup> (mgm. per 100 cc.)          | A 21.2<br>V 21.0   | 21.0  | 24.7        | 20.5<br>24.9    | 25.4 | 24.3 | 22.8 | 22.3 | 21.6         | 20.6<br>21.5 | 20.6  | 20.6<br>20.2   |
| Cl <sup>-</sup> (mgm. per 100 cc.)         | A 340<br>V 338     |       |             | 339<br>355†     |      |      |      |      | 350†         | 339          | 342   | 340<br>342     |
| Specific gravity                           | A 1.057<br>V 1.056 | 1.057 |             | 1.055<br>1.058† |      |      |      |      | 1.057†       | 1.056        | 1.059 | 1.056<br>1.056 |
| Serum analyses                             |                    |       |             |                 |      |      |      |      |              |              |       |                |
| H <sub>2</sub> O (grams per cent)          | A 93.88<br>V 93.88 |       |             | 93.88<br>93.43† |      |      |      |      | 93.58†       | 93.88        | 93.67 | 93.88<br>93.66 |
| K <sup>+</sup> (mgm. per 100 cc.)          | A 17.4<br>V 17.7   |       |             | 17.0<br>22.6†   |      |      |      |      | 17.7†        | 16.8         |       | 17.1<br>17.4   |
| Specific gravity                           | A 1.022<br>V 1.024 |       |             | 1.024<br>1.023† |      |      |      |      | 1.024†       | 1.025        | 1.024 | 1.024<br>1.024 |
| Cell volume                                |                    |       |             |                 |      |      |      |      |              |              |       |                |
| Volume per cent                            | A 42.5<br>V 42.5   |       |             | 41.8<br>44.8†   |      |      |      |      | 44.3†        | 43.1         | 44.0  | 42.7<br>44.1   |
| Calculated erythrocyte content†            |                    |       |             |                 |      |      |      |      |              |              |       |                |
| H <sub>2</sub> O (grams per cent)          | A 65.2<br>V 65.5   |       |             | 65.4<br>64.8†   |      |      |      |      | 66.0†        | 65.6         | 66.1  | 65.2<br>66.2   |
| K <sup>+</sup> (mgm. per 100 grams R.B.C.) | A 24.1<br>V 23.2   |       |             | 22.9<br>26.1    |      |      |      |      | 23.3<br>24.2 |              | 23.0  | 23.0<br>21.6   |

\* The arterial samples were always 40 cc. and were taken slowly throughout the experiment.

† These determinations were run on the pooled remainders of the samples included in brackets after portions had been removed for individual sample analyses.

‡ Calculated by means of the equation:

(Gram % plasma volume-plasma content) + (gram % cell volume · X) = (whole blood content) · 100  
X = cell content in same units as plasma and whole blood contents are expressed

TABLE 2—Concluded

|                                       | REST             |     | STIMULATION |              |      |      |      |      | RECOVERY |            |              |              |
|---------------------------------------|------------------|-----|-------------|--------------|------|------|------|------|----------|------------|--------------|--------------|
| Samples*                              | 1                | 2   | (3          | 4            | 5    | 6)   | 7    | (8   | 9        | 10)        | 11           | 12           |
| Ce. volume venous sample              | 40               | 5   | 5           | 5            | 25   | 5    | 5    | 5    | 5        | 30         | 30           | 51           |
| Duration of period (sec.)             | 410              | 80  | 26          | 22           | 118  | 25   | 21   | 17   | 16       | 92         | 122          | 360          |
| Rate blood flow (cc. per min.)        | 5.9              | 3.8 | 11.5        | 13.6         | 12.7 | 12.0 | 14.3 | 17.7 | 18.8     | 19.6       | 14.8         | 10.2         |
| Calculated values per 100 grams D.W.† |                  |     |             |              |      |      |      |      |          |            |              |              |
| K <sup>+</sup> (mgm. whole blood)     | A 107<br>V 106   | 106 | 118         | 118          | 120  | 116  | 112  | 111  | 107      | 104<br>106 | 103          | 104<br>101   |
| K <sup>+</sup> (mgm. serum)           | A 278<br>V 282   |     |             | 271<br>336   |      |      |      |      | 269      | 268        |              | 272<br>268   |
| K <sup>+</sup> (mgm. R.B.C.)          | A 69.3<br>V 66.7 |     |             | 66.5<br>74.1 |      |      |      |      | 71.3     | 67.8       | 67.1<br>66.5 | 66.5<br>64.3 |
| Cl <sup>-</sup> (grams whole blood)   | A 1.71<br>V 1.71 |     |             | 1.71<br>1.68 |      |      |      |      |          | 1.71       | 1.71         | 1.71<br>1.71 |

† Calculated by means of the equation:

(Volume % content ÷ specific gravity) ÷ (dry weight of 1 gram) = content/100 grams dry weight

(H<sub>2</sub>O)<sub>x</sub> represents the water exchanged with the muscle and erythrocytes per 100 grams of incident serum. The erythrocyte water exchanges are equal to the differences between the calculated serum and whole blood exchanges if these exchanges are expressed per unit of incident blood. The calculated serum and erythrocyte water exchanges for all periods of this experiment are given in table 3. Table 2 includes the erythrocyte water contents calculated by means of the equation:

- (2) (Gram % serum volume · gram serum H<sub>2</sub>O content per gram) +  
(gram % cell volume · X) = gram % whole blood H<sub>2</sub>O content  
X = gram erythrocyte H<sub>2</sub>O content per gram of erythrocytes

*Calculations of chloride and potassium exchanges between blood and muscle.* Chloride and potassium calculations were based on dry weight flows and dry weight concentrations to minimize errors due to water exchanges.

The exchange for any period was calculated from the following equation:

- (3) (Venous flow cc. · (D. W.)<sub>v</sub>) · ((K)<sub>A</sub> - (K)<sub>v</sub>) ÷ 100 =  
mgm. potassium gained or lost by the muscle  
(D. W.)<sub>v</sub> = Dry weight of 1 cc. of venous blood  
(K)<sub>A</sub> = Arterial potassium mgm. per 100 grams dry weight  
(K)<sub>v</sub> = Venous potassium mgm. per 100 grams dry weight

This relation is not strictly valid if the dry weight of the blood passing through the muscle changes. The error introduced by these calculations,

if the water moved as a 2 per cent protein solution, thus resulting in a change in dry weight of the blood, can be shown by calculation to be about one per cent.

TABLE 3  
*Calculated potassium, chloride and water exchanges, Experiment G-6*

| Samples   | REST  | STIMULATION |        |        |        | RECOVERY |        |       |    |
|---|-------|-------------|--------|--------|--------|----------|--------|-------|----|
|   | 1     | 2           | 3-4    | 5      | 6      | 7-8      | 9-10   | 11    | 12 |
| Water exchanges* between blood and muscle in grams    |       |             |        |        |        |          |        |       |    |
| Per 100 Gm.   |       |             |        |        |        |          |        |       |    |
| I.B.:   | -0.70 | -0.64       |        | 5.71   |        | 1.73     | 0.58   | 0.37  |    |
| For period:   | -0.04 | -0.03       |        | 2.56   |        | 0.84     | 0.19   | 0.21  |    |
| Total:  | -0.04 | -0.07       |        | 2.49   |        | 3.32     | 3.50   | 3.71  |    |
| Serum water exchanges in grams                        |       |             |        |        |        |          |        |       |    |
| Per 100 grams   | 0.0   |             |        | 6.85   |        | 4.67     | 3.62   | 2.86  |    |
| I.P.  |       |             |        |        |        |          |        |       |    |
| Per 100 grams   | 0.0   |             |        | 3.63   |        | 2.51     | 1.96   | 1.55  |    |
| I.B.  |       |             |        |        |        |          |        |       |    |
| Erythrocyte water exchanges† in grams                 |       |             |        |        |        |          |        |       |    |
| Per 100 grams   | -0.70 |             |        | 2.08   |        | -0.78    | -1.38  | -1.18 |    |
| I.B.  |       |             |        |        |        |          |        |       |    |
| Potassium exchanges* between blood and muscle in mgm. |       |             |        |        |        |          |        |       |    |
| Per 100 Gm.   |       |             |        |        |        |          |        |       |    |
| D.B.:   | 1.0   | 1.0         | -15.0  | -17.0  | -13.0  | -8.0     | -2.0   | 2.0   |    |
| For period:   |       |             | -0.316 | -0.898 | -0.138 | -0.169   | -0.141 | 0.330 |    |
| Total:  |       |             | -0.32  | -1.21  | -1.35  | -1.52    | -1.66  | -1.33 |    |
| Chloride exchanges* between blood and muscle in mgm.  |       |             |        |        |        |          |        |       |    |
| Per 100 Gm.   |       |             |        |        |        |          |        |       |    |
| D.B.:   | 0.0   |             |        | 30     |        | -20      | 0.0    |       |    |
| For period:   | 0.0   |             |        | 2.53   |        | -1.62    | 0.0    |       |    |
| Total:  | 0.0   |             |        | 2.53   |        | 0.91     | 0.91   |       |    |

I.B. = incident blood, I.P. = incident plasma, D.B. = dry blood.

\* Positive exchange values represent a gain by the muscle.

† Negative exchange values represent a gain by the erythrocytes.

The calculated exchanges of potassium and chloride for this experiment are given in table 3.

The average venous plasma chloride for experiment G-6 was 437 mgm. per 100 grams of serum water. If it is accepted that muscle cells are impermeable to chloride, and that there is a Donnan equilibrium across

the capillary membrane between serum and muscle interstitial-space-chloride, calculations can be made from the chloride and water data which indicate that less than 10 per cent of the water gained by the muscle remained in the interstitial space, and that during stimulation there is a gain of both cellular and interstitial water, but that during recovery the water gain is predominantly cellular.

These calculations are based on rather doubtful assumptions, however, since there is no accurate means of estimating the chloride concentration in the muscle interstitial water under these conditions of rapid water gain and carbon dioxide production by the muscle. The error in measuring small A-V chloride differences is also relatively large. Since these calculations are of doubtful significance the series of equations upon which they are based will be omitted.

Serum potassium exchanges can also be calculated by means of equation (3). In this case the value calculated represents a composite value of the potassium exchanges taking place between serum and muscle and serum

TABLE 4  
*Estimated percentage change in muscle of substances studied (G-6)*

|                       | AVERAGE<br>CONC. PER<br>100 GRAM<br>MUSCLE | ESTIMATED<br>CONTENT<br>IN MUSCLE | GAIN AFTER 4' 31"<br>STIMULATION |          | GAIN AFTER 9' 28"<br>RECOVERY |          |
|-----------------------|--|-----------------------------------|----------------------------------|----------|-------------------------------|----------|
|                       |  |                                   | Amount                           | Per cent | Amount                        | Per cent |
| Water (grams).....    | 76   | 89                                | 2.6                              | 2.9      | 3.6                           | 4.1      |
| Chloride (mgm.).....  | 48   | 56                                | 2.5                              | 4.5      | 0.9                           | 1.6      |
| Potassium (mgm.)..... | 430  | 503                               | -1.4                             | -0.28    | -1.3                          | -0.26    |

and erythrocytes. The erythrocyte potassium exchange should be equal to the differences between the calculated serum and whole blood exchanges if these exchanges are expressed per unit of incident blood. These calculations indicate that 34 per cent of the potassium which was lost by the muscle during stimulation entered the erythrocytes. Erythrocyte potassium content can be calculated directly from the serum whole blood potassium analyses and cell volume determinations by an equation similar to equation (2) given above for calculating erythrocyte water content. These calculated values both on the basis of wet and dry weight are given for this experiment in table 2.

The gastrocnemius preparation weighed 176.5 grams at the start of the experiment; at the end of the experiment the weight had increased to 182 grams, a gain in weight of 5.5 grams during  $2\frac{1}{2}$  hours of perfusion. The muscle alone weighed 117 grams. The estimated concentration changes in the muscle of the substances studied during stimulation and recovery are given in table 4.

**SUMMARIZED RESULTS.** The effect of stimulation on venous blood flow in these experiments is given below:

*Average venous blood flows in cubic centimeters per minute resulting from alternating steady and intermittent tetanic stimulation of from 1½ to 4½ minutes*

| REST     | END OF STIMULATION | 30' OF RECOVERY | 10' OF RECOVERY |
|----------|--------------------|-----------------|-----------------|
| 10(7-17) | 30(13-66)          | 35(18-50)       | 17(9-22)        |

Average weight of muscle: 93 grams (77-118), 7 experiments.

Stimulation in all cases resulted in a marked increase of venous blood flow. The flow doubled or trebled during the period of stimulation. This rapid increase continued for a short period after stimulation and then the flow decreased rapidly so that it usually reached a value within 5 cc. per minute of the resting value within a period of ten minutes after stimulation had ceased. The flow then remained at about this level or decreased slowly until the resting value had been reached about an hour after stimulation. These changes in blood flow are very similar to changes in blood flow obtained by several investigators in human subjects or experimental animals as a result of muscular exercise.

In this case since the perfusion pressure and the venous pressure remain constant and the muscle receives no nervous impulses after the period of stimulation, the increase in flow must be caused by vasodilator substances liberated by the muscle during, and in recovery from, a period of contraction. It should be pointed out that all the blood flowing through the muscle during stimulation and for a period of about ten minutes after stimulation was reserved for chemical analyses. Therefore any vasodilator substances liberated into the blood stream by the muscle during this period were removed from the circulating blood. After this period, the venous blood from the muscle was returned to the venous reservoir. This factor may be of some importance in determining the rapidity of the decrease in the rate of blood flow after stimulation.

**SUMMARY OF WATER EXCHANGES.** The calculated water exchanges of six experiments are summarized graphically in figure 1. Stimulation in all cases resulted in a rapid gain of water by the muscle ranging from about 10 to 20 mgm. per second during the period of most rapid water gain, occurring near the end of and immediately after stimulation. The muscle continued to gain water at a rapid enough rate to be detectable for a period of about ten minutes after stimulation. The rate of gain fell off rapidly, however, during this period and was only a fraction of a milligram per second when ten minutes had elapsed. The rate of water gain after stimulation fell to an undetectable value in only one experiment. In this experiment the A-V water contents were determined 14 minutes after

stimulation had ceased; at this point the A-V difference in water content was negligible.

The prolonged gain of water after stimulation substantiates Boyle and Scott's finding (1938) that severely exercised extremities have an increased volume for long periods of time after the exercise even after eliminating volume increases due to vasodilatation.

Part of this gain in water may be due to an increase in filtration pressure in the capillaries as a result of vasodilatation; however, since the arterial and venous pressures remain constant the increases in capillary perme-

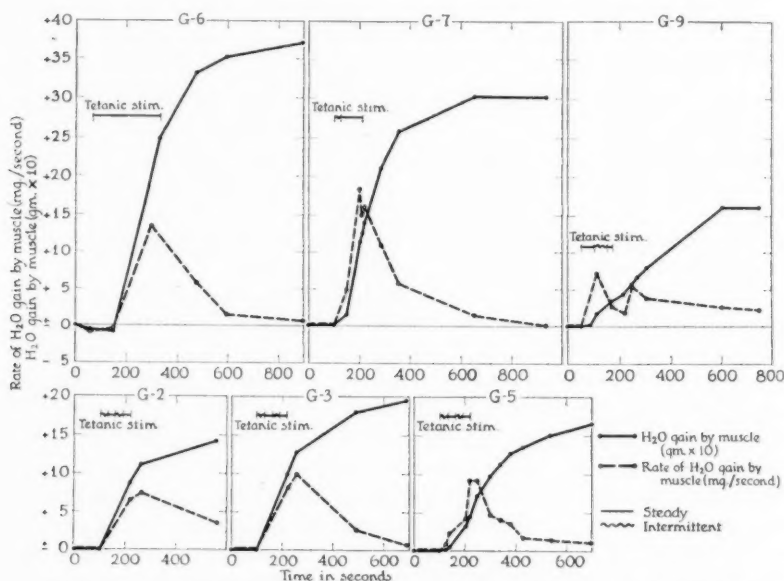


Fig. 1. Water exchanges between dog gastrocnemii and blood during stimulation and recovery.

ability and of osmotically active substances in the muscle must be an even more important factor causing a water gain in this preparation than in the intact animal. The prolonged gain in water is at least an indication of a continued production of osmotically active substances within the muscle after contraction has ceased, since a diffusion lag of water into the muscle of more than ten minutes seems unlikely.

**SUMMARY OF CHLORIDE EXCHANGES.** Complete whole blood chloride analyses were carried out in two experiments. The analyses of one of these experiments is given above in table 2 and the analyses of the other in table 5.

As these tables show, the magnitude of the actual arterial venous differences are small so that the percentage error in determining the difference is correspondingly large.

Calculations based on the assumptions given above indicate that less than one-third of the water gained by the muscle remained in the interstitial space. The results of calculations of differential water gain by the muscle based on these analyses are summarized in figure 2.

Due to the fact that only two experiments of this type have been carried out and as pointed out above the relative errors in determining the A-V differences must have been large the findings are presented with some reservations. The calculations are based on somewhat uncertain assumptions so that the values found for interstitial and cellular water gain may be greatly in error.

It should be pointed out that during muscular contraction when  $\text{CO}_2$  is being produced very rapidly, there must be a relatively large chloride shift taking place from the interstitial spaces to the more strongly buffered

TABLE 5  
*Whole blood chloride analyses, Experiment G-7*

|                  | Cl Mgm. per 100 cc. |        | Cl Mgm. per 100 grams dry weight |        |
|------------------|---------------------|--------|----------------------------------|--------|
|                  | Arterial            | Venous | Arterial                         | Venous |
| Rest.....        | 314                 | 316    | 1.61                             | 1.62   |
| Stimulation..... |                     | 316    |                                  | 1.58   |
| Recovery.....    | 315                 | 319    | 1.62                             | 1.61   |
| Recovery.....    |                     | 318    |                                  | 1.62   |
| Recovery.....    | 314                 | 317    | 1.61                             | 1.62   |

plasma and corpuscles. This fact along with the faster diffusion rate of water with respect to chloride would result in the calculated cellular  $\text{H}_2\text{O}$  gain being fictitiously large and interstitial  $\text{H}_2\text{O}$  gain the opposite.

Figure 2 indicates that during stimulation the water gain by the muscle is both interstitial and cellular, and that the water gain during recovery is nearly all cellular. The  $\text{CO}_2$  production and the chloride diffusion lag would tend to be less during recovery, so that the factors resulting in the calculated cellular  $\text{H}_2\text{O}$  gain being fictitiously large get progressively smaller as recovery progresses. This suggests therefore that the indication that recovery  $\text{H}_2\text{O}$  gain is mainly cellular may be correct.

*Potassium exchanges.* Potassium analyses were carried out in all experiments; in every case stimulation caused an increase in the potassium content of the venous blood. The results of six of these experiments are shown in figure 3.

In the four of these experiments in which the period of stimulation was about two minutes a reversal in the potassium exchange occurred almost

immediately after cessation of stimulation. In experiment G-6 in which the stimulation period was 4 minutes 31 seconds a slight reversal occurred more than 2 minutes after stimulation had ceased.

It should be pointed out that the magnitude of the A-V difference upon which this reversal is based is in some cases within the limits of error of the potassium analyses and usually can only be demonstrated on the basis

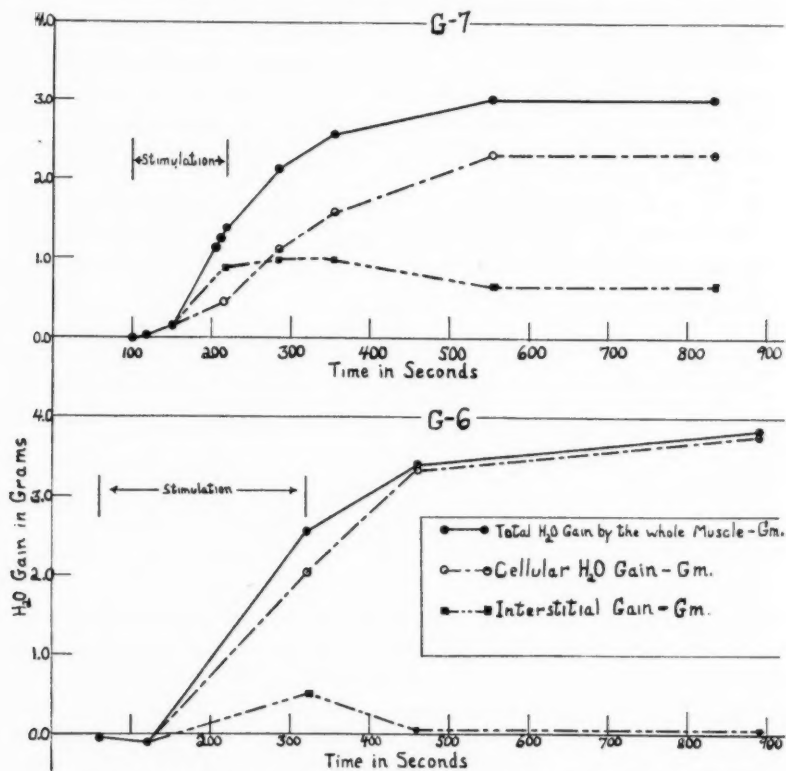


Fig. 2. Calculated gain of water by cellular and extracellular spaces of dog gastrocnemii during stimulation and recovery.

of dry weight; however, the fact that it occurred in every experiment suggests that it is real. The reversal, as demonstrated by this method, is by no means complete. This is undoubtedly due at least in part to the fact that the reversal takes place at a relatively slow rate so that it causes a detectable A-V difference only at the onset. The speed of reversal after short periods of contraction is usually at a maximum almost immediately at the onset of recovery and from this point falls off rapidly to a rate

which cannot be detected by our analyses. The change in the muscle cell from a condition in which it is losing potassium rapidly, as a result of a short period of stimulation, to a condition in which it gains potassium when stimulation ceases must take place almost instantaneously in these cases, since the change is detected by the analyses within 30 seconds. There must necessarily be a relatively long lag in the detection of this change due to the blood contained in the veins and venous cannula and tubing at the end of stimulation, which must have been in contact with contracting muscle.

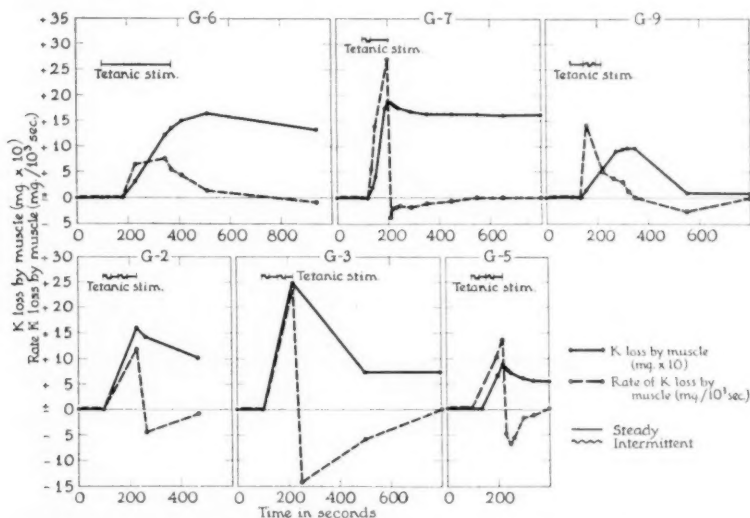


Fig. 3. Potassium exchanges between dog gastrocnemii and blood during stimulation and recovery.

One experiment was carried out in which condenser discharges at the rate of 250 per minute were used as the stimulating current. This muscle gave a faster rate of potassium loss than any of the muscles stimulated with a tetanizing current. This is in confirmation of Fenn (1938), who found that interrupted stimulation was more efficacious than a steady tetanic current for promoting potassium loss from a muscle (table 6).

Two experiments have been carried out to test the hypothesis that the potassium may be liberated at the motor endings supplying the muscle. The same preparation was used as in the above experiments.

*Experiment G-2.* The potassium exchange as a result of 2 minutes of contraction induced by stimulation of the sciatic nerve was determined. Sufficient curare (9 mgm.) was then added to the venous reservoir to abolish the response of the muscle

when the nerve was stimulated. The nerve was then stimulated for three minutes and the potassium exchange determined as before. Electrodes were then placed on the extremities of the muscle and the muscle stimulated directly for three minutes and the potassium exchange again determined. The results are shown in figure 4.

Stimulation of the sciatic nerve before curare and direct stimulation of the muscle after curare caused the ordinary potassium exchange resulting from muscle contraction. Stimulation of the sciatic nerve after curare had no detectable effect on the potassium exchange. A reversal was not detected after direct stimulation probably due to the fact that only one sample was taken during recovery, 6 minutes after stimulation had ceased.

*Experiment G-8.* A preparation was set up using a gastrocnemius muscle the motor nerve to which had been transected aseptically 13 days previously. The muscle

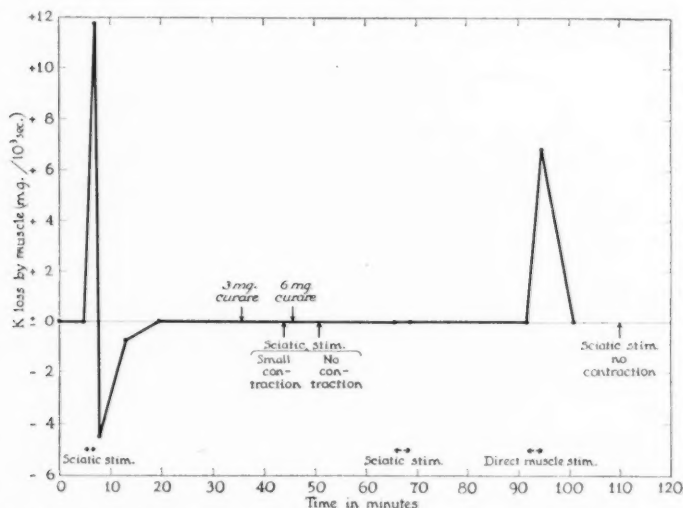


Fig. 4. Rate of potassium exchange between the dog gastrocnemius and blood during stimulation before and after curarization.

was stimulated directly by means of electrodes placed upon the extremities of the muscle. The muscle lost potassium during stimulation similarly to a normal muscle (table 6).

In table 6 the experiments are arranged in order of the rate of maximum blood flow during the stimulation period. This table indicates that the rate of potassium loss by the muscle varies directly with the rate of blood flow. It may be noted that the potassium A-V difference is fairly constant and nearly independent of the rate of blood flow. The paucity of critical experimental data on this point precludes the possibility of a final interpretation of this relationship. The fact that interrupted stimulation is more efficacious than continuous stimulation in promoting potassium loss

from a muscle might be explained on the basis of effects upon blood flow. It has been shown by Fenn (1938) that a muscle loses less potassium during stimulation if the blood flow to the muscle is restricted. In a recent paper Fenn (1939) has indicated that when a hind limb is caused to lose small amounts of potassium by severe restriction of the arterial blood flow to it, the arterio-venous potassium difference is independent of the degree of asphyxia even when the blood flow is shut off completely.

*Erythrocyte exchanges.* The changes in the erythrocytes as calculated from cell volume, whole blood, and serum analyses were according to

TABLE 6  
*Relation of rate of blood flow to rate of potassium loss*  
Maximum values during stimulation period\*

| EXPERIMENT | VENOUS<br>BLOOD FLOW | RATE K LOSS           | ARTERIAL VENOUS POTASSIUM DIFFERENCE IN<br>MGM. PER 100 GRAMS OF |                              |                        |
|------------|----------------------|-----------------------|--|------------------------------|------------------------|
|            |                      |                       | Whole blood<br>dry weight  | Whole blood H <sub>2</sub> O | Serum H <sub>2</sub> O |
|            | cc. per min.         | mgm. per 1000<br>sec. |  |                              |                        |
| G-8†       | 8.2                  | 5.6                   | 23.1   | 6.0                          |                        |
| G-4        | 11.9                 | 7.7                   | 21.7   | 5.2                          |                        |
| G-6        | 14.0                 | 7.7                   | 17.0   | 6.0                          | 6.1                    |
| G-9        | 16.4                 | 14.2                  | 24.0   | 6.7                          | 7.6                    |
| G-5        | 26.0                 | 13.3                  | 13.4   | 3.7                          |                        |
| G-2        | 28.0                 | 12.0                  | 19.7   | 4.4                          |                        |
| G-3        | 45.0                 | 24.0                  | 19.0   | 5.0                          | 4.4                    |
| G-7        | 45.0                 | 27.0                  | 20.2   | 5.6                          | 6.9                    |
| G-10‡      | 68.0                 | 44.1                  | 25.2   | 7.6                          | 6.7                    |

\* Average period of indirect intermittent or steady tetanic stimulation: 149 (49-271) seconds.

† The muscle was stimulated directly, the sciatic nerve having been transected 13 days previously.

‡ The muscle was stimulated indirectly by means of condenser discharges at the rate of 250 per minute.

changes that could have been predicted as resulting from increased venosity of the blood due to muscular contraction.

The venous cell volume increased as much as 2 per cent during stimulation and remained increased during the first 10 or 15 minutes of recovery. The increase in cell volume in this case is due to two factors: most important the relatively large loss of water by the plasma to the muscle, and secondly the tendency of the corpuscles to gain water when blood becomes more venous.

The water content of the erythrocytes increased during stimulation in every experiment except G-6. The rate of water gain by the muscle in proportion to blood flow was more than twice as great in this experiment

as in any of the others. It seems probable that in this case the loss of water by the blood was great enough to overbalance the tendency of the corpuscles to gain water due to increased vascosity so that an actual loss of erythrocyte water resulted. Erythrocyte water exchanges were calculated in all cases both by cell volume and A-V plasma, whole blood water contents, and by the differences in calculated plasma and whole blood water exchanges.

Complete plasma and whole blood chloride analyses were carried out in only two experiments. In those experiments the venous red cell chloride content increased during stimulation as would be predicted from the chloride shift.

The venous erythrocyte potassium content calculated on a wet or dry weight basis increased during stimulation in every case.

It should be pointed out that error in erythrocyte concentrations as calculated from analyses of plasma and whole blood and cell volume determinations is relatively large. Therefore the significance of small changes is doubtful; however, the uniformity of finding increased erythrocyte potassium during stimulation when potassium was being given off to the blood quite rapidly suggests that this increase is real. The serum was not separated from the erythrocytes until about 30 minutes after collection of the sample so that changes found in erythrocyte content may have no bearing on the erythrocyte exchanges which take place when the blood is passing through the muscle.

**DISCUSSION.** The contributions of these experiments to the field of electrolyte exchanges in relation to muscular exercise can be summarized as follows:

The development of a method for studying potassium and water exchanges between blood and muscle by means of which it is possible with considerable accuracy to determine the time relations and the magnitude of potassium exchanges of less than 1 mgm. (0.5 per cent of the total potassium content of the muscle) and water exchanges of less than 1 gram (1 per cent of the total  $H_2O$  content of the muscle) during short periods of stimulation and recovery. It would be impossible to detect such small exchanges by means of paired muscle analyses.

The results of these experiments confirm in principle the studies of Fenn and other recent investigators. The earlier researches, however, offered little information concerning the time relations of these exchanges.

In this study the output of potassium by muscle began almost immediately at the onset of contractions. Therefore, it appears very probable that potassium loss is a normal characteristic of all striated muscle contractions, and is not merely a characteristic of prolonged contractions and muscular fatigue, as has been suggested by earlier investigators.

Since the potassium and water exchanges definitely do not run parallel

it seems probable that they result from different mechanisms. Fenn (1938) has previously cited some evidence pointing to this fact.

The recovery of the potassium lost during stimulation occurred much more rapidly in these experiments than Fenn has been able to demonstrate by means of paired muscle analyses. This may be due to a real difference since it does not seem improbable that long periods of stimulation of 30 minutes or more, such as Fenn used, may alter the muscle cells in some way so that recovery of the lost potassium is delayed.

The failure to demonstrate a reversal of the water gain during the recovery period from a period of contraction may be due to two factors: 1, as these experiments suggest and in agreement with previous researches, the loss of the water gained by the muscle may not occur for relatively long periods of time after contraction has ceased. No attempt was made to follow recovery exchanges for periods of more than 15 minutes after cessation of contraction in these studies, since it is probable that the exchanges occurring after this period takes place at too slow a rate to produce detectable A-V differences. 2. A heart lung perfused gastrocnemius muscle is not a normal muscle, and it is not impossible that the recovery processes in such a muscle are interfered with, especially since all of these preparations gain some weight.

The potassium loss resulting from direct stimulation of a curarized muscle and a muscle whose motor nerve ending were degenerated is convincing proof that the potassium liberated during muscular contraction does not arise solely from the motor nerve endings.

The failure to find a potassium loss from a curarized muscle as a result of stimulating the motor nerve to the muscle does not agree with Reginster's (1938) finding of an increased "diffusible potassium" content in frog muscle under these conditions. The two experiments are not comparable, however, so this experiment can not be considered actual disproof of Reginster's results.

These experiments give little if any evidence as to the actual physico-chemical phenomenon responsible for these potassium and water exchanges. Since the various mechanisms which have been proposed to explain the known facts are not widely accepted it seems unprofitable at this stage of knowledge to discuss this phase of the problem. There are some indications however that potassium loss by the muscle is not a simple "liberation" such as might result from increased permeability of the cell membrane but may be due to a shift in the concentration levels of the "steady state" which may normally exist between intracellular and extracellular potassium.

#### SUMMARY

1. A heart lung gastrocnemius preparation is described. By means of this preparation it is possible to determine with reasonable accuracy both

the magnitude and time relations of relatively small electrolyte and water exchanges between blood and muscle during stimulation and recovery by chemical analysis of arterial and venous blood.

2. Indirect tetanic stimulation of the muscle results in:

a. A trebling of the blood flow through the muscle which decreases rapidly toward the resting value in the first ten minutes of recovery and then levels off so that the resting blood flow, if attained, is not reached for more than 45 minutes after stimulation has ceased.

b. A rapid water gain by the muscle which continues at a progressively slower rate for about ten minutes after stimulation has ceased.

c. A gain of chloride by the muscle indicating an increase in the interstitial space of the muscle which according to calculations amounts to less than one-third of the total water gained by the muscle and occurs chiefly in the period of stimulation.

d. A rapid loss of potassium by the muscle during stimulation, followed usually immediately after stimulation by a reversal which results in a partial recovery by the muscle of the potassium lost.

e. An increased cell volume, a gain in erythrocyte potassium and chloride during stimulation, and water, unless the loss of water by the blood to the muscle is rapid enough to overcome the shift of water into corpuscles due to increased venosity of the blood.

3. Stimulation of the motor nerve to a curarized muscle does not cause potassium exchanges detectable by this method.

4. Direct stimulation of a normal muscle, a curarized muscle, and a muscle whose motor nerve has been transected 13 days previously result in potassium changes similar to those elicited by indirect stimulation of a normal muscle. Therefore potassium loss by striated muscle during contraction is not dependent on the presence of functional motor nerve endings.

5. Potassium and water exchanges resulting from stimulation and recovery of striated muscle do not run parallel.

6. The rate of potassium loss by the muscle during stimulation appears to vary directly with the rate of blood flow through the muscle.

7. It is probable that potassium loss and water gain by striated muscle is a characteristic of all normal muscular contractions.

#### REFERENCES

- BANUS, M. G., L. N. KATZ AND J. W. MULL. *This Journal* **81**: 628, 1936.  
BOYLE, R. W. AND F. H. SCOTT. *This Journal* **122**: 569, 1938.  
BREH, F. AND O. H. GAEBLER. *J. Biol. Chem.* **87**: 81, 1930.  
FENN, W. O. *Physiol. Rev.* **16**: 450, 1936.  
*This Journal* **124**: 213, 1938.  
*Ibid.* **128**: 139, 1939.  
PETERS, H. C. AND M. B. VISSCHER. *Am. Heart J.* **11**: 273, 1936.  
REGINSTER, A. *Arch. Internat. de Physiol.* **45**: 69, 1937.  
*Ibid.* **47**: 24, 71, 1938.  
VAN SLYKE, D. D. AND J. SENDROY, JR. *J. Biol. Chem.* **58**: 523, 1923.

## UTERINE CHANGES IN THE RABBIT WITH THE ADVENT OF PREGNANCY<sup>1</sup>

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The respiratory metabolism of rodent uteri has been examined during various stages of the oestrous cycle and pregnancy (1), (10), (9) and under the influence of ovarian hormone injection (11), (12), (16). During the period of blastocyst growth in the rat the acidity of the endometrial tissue rises (5), and in the rabbit the ascorbic acid concentration increases to a maximum (15). According to the latter authors the ascorbic acid content of the corpora lutea and the urinary ascorbic acid also increase during this period.

It was considered desirable to attempt some correlation of these factors by measuring simultaneously: 1, the concentrations of ascorbic acid and other reducing substances; 2, the oxygen uptake, and 3, the concentration of the respiratory enzyme, cytochrome oxidase in uteri of mature rabbits in various stages of the reproductive cycle, and of immature rabbits brought to sexual maturity by the injection of gonadotropic material. Concomitantly the content of the reducing substances was measured in the ovaries, adrenals, and pituitaries of a number of the experimental animals. The animals receiving gonadotropic hormone injection were ordinarily sacrificed at 15 to 18 hours after an intravenous ovulating injection that was preceded by three days of subcutaneous injection (see Pincus, 14).

*Ascorbic acid and glutathione determinations.* The weighed tissues are extracted with 4 per cent sulfosalicylic acid, one aliquot titrated for ascorbic acid with 2, 6, dichlorophenol-indophenol and for GSH with 0.0001N  $\text{KIO}_3$ , 5 per cent KI and soluble starch (18) (17). Sulfosalicylic acid was used in preference to trichloroacetic and metaphosphoric acids because the latter interferes with the iodate titration. With our solutions 1 mgm. ascorbic acid was equal to 5.80 cc. of the dye and 2.00 cc. of the 0.0001N  $\text{KIO}_3$ , and 1 mgm. of glutathione reduced 0.70 cc. of the  $\text{KIO}_3$  solution. Freshly dissected tissue was used for these determinations although we find that uterine tissue kept for 24 hours in the refrigerator gives the same

<sup>1</sup> Aided by grants from the Rockefeller Foundation and the National Research Council Committee for Problems of Sex. Works Progress Administration Project no. 665-14-3-766.

values as freshly dissected uterus; the other tissues employed, especially the pituitaries, do not, however, retain reducing activity on storage in the cold.

In order to be certain that the 2, 6, dichlorophenol-indophenol titrations measure ascorbic acid concentration only and not other reducing substances we prepared the specific ascorbic acid oxidase from summer squash as described by Graubard (3), and treated aliquots of various tissue extracts with 0.5 cc. of the enzyme solution at pH 5.0 for 10 minutes. The aliquots were reacidified and titrated against the dye and  $\text{KIO}_3$  with the results shown in table 1. These data demonstrate conclusively that in the tissues used the enzyme oxidizes only the ascorbic acid and that the

TABLE 1  
*Titration of mixtures of crystalline ascorbic acid and glutathione compared with titrations of tissue extracts*

| SOLUTION TITRATED  | WITHOUT ENZYME |                | AFTER TREATMENT WITH ENZYME |                |
|--|----------------|----------------|-----------------------------|----------------|
|  | Dye            | $\text{KIO}_3$ | Dye                         | $\text{KIO}_3$ |
|  | cc.            | cc.            | cc.                         | cc.            |
| Crystalline ascorbic acid, 0.1 mgm. ....   | 6.22           | 2.02           | 0.14                        | 0.14           |
| Crystalline glutathione, 0.1 mgm. ....   | 0.08           | 0.70           | 0.10                        | 0.68           |
| 0.05 mgm. ascorbic acid + 0.05 mgm. glutathione. ....  | 3.24           | 1.34           | 0.08                        | 0.52           |
| 4 per cent sulfosalicylic extract from ovary, 1 cc. ....                                       | 1.52           | 2.40           | 0.12                        | 1.70           |
| 4 per cent sulfosalicylic extract from adrenals, 1 cc. ....                                    | 3.44           | 1.50           | 0.12                        | 0.40           |
| 4 per cent sulfosalicylic extract from uterus (injected, ovulating and stimulated), 1 cc. .... | 0.92           | 1.02           | 0.10                        | 0.66           |
| 2 pit. 0.65 gram, 1 cc. ....   | 0.70           | 0.55           | 0.08                        | 0.30           |

other  $\text{KIO}_3$  reducing substances (presumably glutathione or other SH compounds) do not affect the dye titration significantly.

Table 2a gives the results obtained with regard to concentration of ascorbic acid and glutathione in various tissues of differently treated rabbits. The glutathione is calculated by subtracting the iodine value of the ascorbic acid from the total and dividing by the glutathione equivalent. The individual measurements of both reducing substances show considerable variation often exceeding 100 per cent. The average values given indicate, however, that injected rabbits as well as those pregnant three days show a definite increase in ascorbic acid content of their uteri with only a slight increase in the glutathione content. A similar increase is shown in the ascorbic acid and glutathione values of the adrenals of pregnant animals. The latter show no remarkable change in pituitary reducing

substances, but the injected rabbits consistently demonstrated an increase in pituitary ascorbic acid. The values for the ovaries are almost constant probably because they vary with regard to corpora lutea which have been shown to possess increased concentrations of ascorbic acid (15).

TABLE 2a

*Titration of ascorbic acid and total reducing substances*

Titration values of extracts of various tissues from female rabbits in stated conditions. The figures in this table express milligrams of ascorbic acid or glutathione per gram of tissue, as obtained from titration with 2-6 dichlorophenol-indophenol and 0.0001N KIO<sub>3</sub>, respectively. The figures in table 2 (b) express cubic millimeters of oxygen per milligram dry weight of tissue, dry weight ratios and units of cytochrome oxidase per cubic centimeter of extract. All enzyme extractions made with 20 cc. of M/15 phosphate buffer pH 7.3 per gram of uterine tissue.

| CONDITION OF ANIMALS                                       | UTERUS        |              | ADRENALS      |              | OVARIES       |              | PITUITARY     |              |
|--|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|
|  | Ascorbic acid | Gluta-thione | Ascorbic acid | Gluta-thione | Ascorbic acid | Gluta-thione | Ascorbic acid | Gluta-thione |
|  | mgm.          | mgm.         | mgm.          | mgm.         | mgm.          | mgm.         | mgm.          | mgm.         |
| Normal, young 3-5 mos. same breed as those injected (3) .. | 0.135         | 0.660        | 1.280         | 1.260        | 0.345         | 1.140        | 0.540         | 2.520        |
| Injected, not ovulating, stimulated (3) ..                 | 0.130         |              | 1.620         |              | 0.240         |              | 1.130         |              |
| Injected, not ovulating, not stimulated (2) .....          | 0.103         |              | 1.056         |              | 0.348         |              | 0.890         |              |
| Injected, ovulating, stimulated (10) ....                  | 0.168         |              | 1.502         |              | 0.222         |              | 0.908         | 2.526        |
| Injected, immature superovulating 3 days pregnant (3) ..   | 0.175         |              | 1.330         |              | 0.158         |              | 1.060         |              |
| Normal, mature rabbits (5) .....                           | 0.090         | 0.588        | 0.694         | 0.873        | 0.274         | 1.206        | 0.340         | 1.323        |
| 3 days pregnant (4) ..                                     | 0.164         | 0.687        | 1.155         | 2.580        | 0.330         | 1.560        | 0.330         | 1.620        |
| 6 days pregnant (4) ..                                     | 0.109         | 0.558        | 0.820         | .750         | 0.222         | 1.284        | 0.407         | 0.984        |
| 12 days pregnant (3) ..                                    | 0.090         | 0.390        | 0.835         | 1.320        | 0.265         | 0.930        | 0.395         | 1.710        |
| 19 days pregnant (3) ..                                    | 0.078         | 0.357        |               |              |               |              | 0.360         | 1.635        |
| 28 days pregnant (4) ..                                    | 0.092         | 0.333        | 0.590         | 0.780        | 0.255         | .885         | 0.245         | 1.165        |

The glutathione content of pregnant uteri seems to show a diminution after the sixth day of pregnancy. Generally speaking the concentrations of the two reducing substances run parallel in all tissues. Their ratios vary, however, in the organs examined though they are fairly constant for each specific tissue.

*The respiration of uterine tissue.* Simultaneously with the above determinations of ascorbic acid and glutathione contents of the uterus, the

oxygen uptake of uterine tissue was also followed. This was done in Barcroft-Warburg respirometers of vessel capacity about 18 cc.

The uterus of the rabbit was slit along the line of attachment of the omentum, the wall flattened on a sterile towel and strips of endometrium less than 0.5 mm. in thickness cut lengthwise with sharp scissors, care being taken not to include any parts of the muscular wall. These strips are referred to in table 2b as endometrium. The tissue is rapidly weighed (on a Roller-Smith Precision Balance) and transferred to the respirometer vessel containing 2.8 cc. M/15 phosphate buffer pH 7.3 in

TABLE 2b  
*Oxygen uptake and enzyme content*

| CONDITION OF ANIMALS  | ENDO-<br>METRIUM           | D.W.<br>W.W.<br>PER CENT | WHOLE<br>UTERUS            | D.W.<br>W.W.<br>PER CENT | CYTO-<br>CHROME<br>OXIDASE |
|---|----------------------------|--------------------------|----------------------------|--------------------------|----------------------------|
|   | <i>c.mm. O<sub>2</sub></i> |                          | <i>c.mm. O<sub>2</sub></i> |                          | <i>units</i>               |
| Normal, young 3-5 mos. same<br>breed as those injected (3)..... | 4.35                       | 9.5                      | 2.48                       | 15.5                     | 1.2                        |
| Injected, not ovulating, stimu-<br>lated (3).....               | 5.02                       | 10.1                     | 2.57                       | 13.1                     | 1.3                        |
| Injected, not ovulating, not stimu-<br>lated (2).....           | 4.85                       | 9.1                      | 2.10                       | 12.9                     | 1.0                        |
| Injected, ovulating, stimulated<br>(10).....                    | 4.40                       | 8.7                      | 2.05                       | 13.1                     | 1.1                        |
| Normal, mature rabbits (5).....                                 | 4.35                       | 9.6                      | 2.40                       | 16.2                     | 1.4                        |
| Injected, mature superovulating<br>3 days pregnant (3).....     | 7.65                       | 6.90                     | 3.20                       | 11.1                     | 1.8                        |
| 3 days pregnant (4).....  | 7.85                       | 8.7                      | 3.38                       | 12.7                     | 2.3                        |
| 6 days pregnant (4).....  | 5.40                       | 10.3                     | 2.65                       | 13.9                     | 1.8                        |
| 12 days pregnant (3).....                                       | 4.80                       | 9.9                      | 3.24                       | 13.0                     | 1.7                        |
| 19 days pregnant (3).....                                       | 5.40                       | 7.1                      | 3.30                       | 11.5                     | 1.2                        |
| 28 days pregnant (4).....                                       | 6.40                       | 7.5                      | 2.87                       | 11.5                     | 1.4                        |

the outer chamber and 0.2 cc. 20 per cent KOH (or NaOH) in the inner chamber in which pieces of filter paper are also immersed.

The oxygen uptake of whole uterus was also measured. This type of tissue was obtained by cutting long strips of uterus along the slit surface, care being taken to maintain a uniform thread-like shape of the slices. These strips contain both myometrium and endometrium.

All experiments were performed at  $37.5^{\circ}\text{C.} \pm 0.05^{\circ}\text{C.}$  The vessels were allowed to equilibrate 15 minutes and measurements were taken for two hours or longer. The oxygen uptake curve is always a straight line in a plot of oxygen consumed against time. Within the limits of our experimental procedures the rates and amounts of oxygen taken up are directly proportional to amounts of uterine tissue used.

The oxygen uptake of uterine tissues is expressed in cubic millimeters

per milligram dry weight. After completion of the respiration experiment, the tissues are removed from the vessels, dipped quickly in distilled water to wash them of buffer and placed on a porcelain plate designed for indicator tests. This plate with the tissues is kept in an oven at 106-110°C. overnight and the cooled tissues weighed the next day.

The M/15 phosphate buffer medium used in our experiments seems as satisfactory as serum. The rates of oxygen uptake for the first two or three hours are rectilinear with time in both cases and show no tendency to fall off until after six hours' duration. Table 3 shows the rates of oxygen uptake in buffer and serum, demonstrating that either one is equally satisfactory. It should be noted that changes in pH of buffer exceeding 0.2 unit lead to irregularities in the oxygen uptake curve.

TABLE 3

*Uterine tissue respiration in serum and M/15 phosphate buffer pH 7.3*

Figures refer to cubic millimeters of oxygen per milligram dry weight of tissue tested. Percentage dry weight is given for comparison of the two tissues employed.

| CONDITION OF RABBIT                   | O <sub>2</sub> UPTAKE OF<br>ENDOMETRIUM |                            | PER CENT<br>D.W. | O <sub>2</sub> UPTAKE OF<br>WHOLE UTERUS |                            | PER CENT<br>D.W.     |
|---------------------------------------|---|----------------------------|------------------|--|----------------------------|----------------------|
|                                       | Buffer                                  | Serum                      |                  | Buffer                                   | Serum                      |                      |
|                                       | <i>c.mm. O<sub>2</sub></i>              | <i>c.mm. O<sub>2</sub></i> |                  | <i>c.mm. O<sub>2</sub></i>               | <i>c.mm. O<sub>2</sub></i> |                      |
| 5 days pregnant.....                  | 4.45                                    | 4.90                       | 10.3<br>12.4     | 2.91<br>2.31                             | 2.85                       | 12.2<br>11.5<br>12.5 |
| Superovulating unferti-<br>lized..... | 4.05                                    | 3.90                       | 12.0<br>12.6     | 2.40                                     | 2.22                       | 15.0<br>15.3         |
| Injected not ovulating....            | 3.50                                    | 3.60                       | 10.0<br>9.8      | 1.50                                     | 1.55                       | 13.8; 14             |

The results of the experiments on respiration are summarized in table 2b. They indicate that endometrium invariably has a rate of oxygen uptake about twice as high as whole uterus strips. Myometrium alone shows a slightly lower respiration than whole uterus, due presumably to the fact that the amount of endometrium in whole uterus strips is a very small fraction of the total. The values for percentage dry weight of endometrium and whole uterus prove that the higher respiration of the former cannot be due to its higher water or blood contents alone.

It should be noted that the respiration values of endometrium and whole uterus show relatively little variation from animal to animal within the same group. Differences seldom exceed 20 per cent. For this reason the higher values obtained for the respiration of endometrium and whole uterus of the three day pregnant rabbits and the injected and mated animals seem to be significant, particularly since the rise is visible in each individual case.

Experiments have also been performed to determine the respiratory quotients of uterine tissues by the method of differences (2). Several stimulated and non-stimulated uteri were tested and the respiratory quotient was found to vary from 0.9 to 1.1.

We have also measured the respiration of uterine tissues in the presence of several inhibiting or possibly accelerating substances. It can be seen from the data presented in table 4 that cyanide, iodoacetate and salicylaldehyde produce definite inhibition while the other substances listed do not seem to show any definite effect on the respiration. On the whole it may be stated that in the total respiration of the uterine tissues, oxidative and glycolytic processes are involved.

TABLE 4  
*Effect of addition of various substances upon uterine respiration in M/15 phosphate buffer at pH 7.3*

| CONDITION OF RABBIT                  | TISSUE       | RESPIRATION OF CONTROL     | SUBSTANCE         | RESP. IN ITS PRESENCE      |
|--------------------------------------|--------------|----------------------------|-------------------|----------------------------|
|                                      |              | <i>c.mm. O<sub>2</sub></i> |                   | <i>c.mm. O<sub>2</sub></i> |
| Injected, non ovul.....              | Whole uterus | 1.50                       | Cytochrome        | 2.05                       |
| Normal.....                          | Endometrium  | 3.50                       | Cytochrome        | 4.60                       |
| 3 days pregnant.....                 | Whole uterus | 3.80                       | Cytochrome        | 2.70                       |
| Injected, not ovul.....              | Whole uterus | 2.50                       | Urethane, 2 mgm.  | 2.39                       |
| Normal.....                          | Whole uterus | 2.55                       | Succinate, 2 mgm. | 3.90                       |
| Normal.....                          | Endometrium  | 3.60                       | Succinate         | 3.55                       |
| 6 days pregnant.....                 | Endometrium  | 6.00                       | Iodoacetamide     | 2.7                        |
| Injected, not stim.....              | Endometrium  | 3.4                        | Iodoacetamide     | 0.6                        |
| Injected.....                        | Whole uterus | 3.80                       | Glucose           | 3.40                       |
| 3 days pregnant.....                 | Endometrium  | 4.90                       | Salicylaldehyde   | 2.70                       |
| Injected, mated 3 days pregnant..... | Whole uterus | 3.20                       | Thiourea          | 3.40                       |
| Injected, ovulating.....             | Endometrium  | 5.4                        | KCN, 0.01N        | 0.6                        |

*Concentrations of cytochrome oxidase.* Experiments were also conducted to determine whether the uterine changes in pregnancy and in consequence of pituitary extract stimulation were at all correlated with changes in the cytochrome oxidase-cytochrome system. Preliminary experiments had shown that the enzyme was present in uterine tissue though its method of extraction was different from the widely accepted extraction of oxidase from heart muscle as given by Keilin (6) and Ogston and Green (13). When fresh uterine tissue which shows the normal rate of oxygen uptake is ground and suspended in buffer or serum, it will show no oxygen uptake in the respirometer, even if such substrates as glucose, or sodium succinate are added. On the addition of p-phenylene diamine a slow uptake of oxygen is noted. Yet this suspension contains high concentrations of cytochrome oxidase as can be shown by mixing some of it with p-phenylene

diamine and cytochrome. Its failure to oxidize p-phenylene diamine rapidly, except when cytochrome is added, must be taken to mean that the uterus contains extremely little cytochrome, though much enzyme.

Washing the tissue with water in the manner in which cytochrome oxidase is prepared from heart muscle tends at times to inactivate the enzyme. In our method of extraction the tissue is freed from blood and is ground with M/15 phosphate buffer pH 7.30 until a homogeneous brei is obtained. This is centrifuged fifteen minutes at 2500 r.p.m. and the supernatant solution filtered. The resulting solution is clear, but opalescent and contains the enzyme. All extractions were made with 20 cc. of buffer per gram of uterus. The cytochrome was prepared by the method of Keilin and Hartree (8). The activity of the oxidase extract was determined by mixing in respirometer vessel p-phenylene diamine (4 mgm.), cytochrome uterine extract and buffer to make a total volume of 2.9 cc., each vessel containing in addition 0.1 cc. 20 per cent KOH in the central chamber. The substrate was always put in the side arm. The bath was kept at 37.0°C. and the mixtures equilibrated for 10 to 15 minutes. A reading was taken after 10 minutes and the substrate then mixed with the other components. The oxygen uptake against time in minutes is rectilinear for varying amounts of enzyme, within limits, and the slope of the line is proportional to amounts of enzyme added. With higher enzyme concentrations no increase in velocity is noted. A similar relation holds for cytochrome (7) (3).

Our measurements of oxidase concentrations of uteri in various stages of the sex cycle were taken with optimum cytochrome and oxidase concentrations deduced from the above considerations. The results are given in the last column of table 2 (b). Enzyme concentration is expressed in terms of units according to the method of Graubard and Nelson (4). One unit represents here the amount of enzyme which in the presence of excess of cytochrome and 4 mgm. of p-phenylene-diamine in a total volume of 3.0 cc. at pH 7.3 and at a temperature of 37°C. in M/15 phosphate buffer, will result in an uptake of 100 c.mm. of oxygen per hour. All extractions were made with 20 cc. of buffer per gram of uterus.

The results seem to show that no significant changes in the cytochrome oxidase concentration occur with the onset of pregnancy, with the exception of the three days pregnant animals. The uteri of these animals gave uniformly higher yields as did also the uteri of stimulated and mated rabbits from which uteri were excised three days after mating.

Tests were made for the presence of succino-dehydrogenase in our uterine preparations. This enzyme was found to be weakly active in the uncentrifuged suspensions in which the cytochrome oxidase is about four to six times more active than in the centrifuged preparations. In the latter no trace of succino-dehydrogenase can be detected.

The increased enzyme concentration in the uteri of three days pregnant animals, both normal and injected, in which the oxygen uptake is also raised, as well as the concentration of ascorbic acid and total reducing substance, may suggest some correlation between these processes. Yet some contradictory evidence must be taken into account. Thus, when uteri are kept in the cold at 6°C. for 48 hours in covered Petri dishes the rate of oxygen uptake of intact tissue invariably falls to about one-half of its original, some decline is sometimes noted in the oxidase content, though more often the concentration does not vary, while the ascorbic acid and reducing substance content invariably remain constant. The following experiment may serve as an illustration. After 48 hours uterine endometrium respiring immediately after excision at the rate of 4.80 c.mm. oxygen per mgm. dry weight, showed a rate of 2.45. The percentage dry weight rose from 9.2 to 15.0. The concentration of oxidase was 1.4 units per cc. while at the beginning it was 1.6 units, not a significant difference. Yet the values for ascorbic acid per gram of tissue were: uterus 0.150, adrenals 1.740, ovaries 0.320 and pituitary 0.800 mgm. in each case the same as immediately after excision.

**DISCUSSION.** The three properties of the uterus, the ascorbic acid and glutathione concentrations, tissue respiration and cytochrome oxidase content were selected for study because of their possible rôles as physiological indicators. In the case of oxygen uptake from air there is a definite and consistent increase on the third day of pregnancy. The increase in the case of oxidase is small but definite. Yet too many factors enter the process of extracting the enzyme and determining its concentrations to give the results more than suggestive value. On the other hand the ascorbic acid measurements yield wide sample variability so that the increase shown by the tissues of three days pregnant animals may not be significant in view of the numbers used. The fact that all these features show increases on the third day of pregnancy may indicate that they express heightened physiological activity during that period.

There exists the possibility that a survey of as many specific physiological systems within uterine tissue as can be isolated will not only reveal variables which can serve as indicators of changes that set in with pregnancy, but also that these reactions may be affected by interaction with various hormones. In this interaction either the hormone or the selected physiological reaction, or both, may be modified. A study of the existence of such modifications and of their nature may serve to elucidate the physiological mechanism of the mode of hormone action.

#### SUMMARY

1. Young female rabbits (3-6 mos. old) injected with pituitary extracts show an increase in the concentration of ascorbic acid in their pituitaries.

2. Evidence is presented to show that the reducing substance of all the tissues tested—uterus, ovary, adrenal and pituitary—are composed of ascorbic acid and glutathione only.

3. All these organs show in the three-day pregnant animals somewhat increased concentrations of ascorbic acid and glutathione. During the subsequent days of pregnancy the concentrations decline, reaching normal level by the twelfth day.

4. The rate of oxygen uptake of whole uterus and endometrium is constant for several hours in serum or M/15 phosphate buffer medium of pH 7.3. This respiration rate is about 100 per cent higher for endometrium than whole uterus and 150 per cent than myometrium.

5. In 3-day pregnant rabbits the respiration rates of endometrium and whole muscle show an increase of 75 per cent above the rates of these tissues in non-pregnant rabbits. Uteri in animals during subsequent stages of pregnancy do not maintain this increase though they respire at a rate somewhat higher than that of normal uteri.

6. Uterine tissue contains very active concentrations of cytochrome oxidase but extremely small amounts of cytochrome. The oxidase concentration of uterine tissue shows an increase on the third day of pregnancy. During the remaining days the enzyme concentration is only slightly higher than normal.

7. The cytochrome oxidase of rabbit uteri is usually inactivated by washing tissue with water, thus differing from beef heart cytochrome oxidase.

8. The value of this approach to the study of hormone action is discussed.

#### REFERENCES

- (1) DAVID, J. C. *J. Pharmacol. and Exper. Therap.* **43**: 1, 1931.
- (2) DIXON, M. *Manometric method*. Cambridge, 1934.
- (3) GRAUBARD, M. *Enzymol.* **5**: 332, 1939.
- (4) GRAUBARD, M. AND J. M. NELSON. *J. Biol. Chem.* **112**: 135, 1935.
- (5) HALL, B. V. *Physiol. Zool.* **9**: 471, 1936.
- (6) KEILIN, D. *Proc. Roy. Soc.* **104**: 206, 1929.
- (7) KEILIN, D. *Proc. Roy. Soc. B.* **106**: 418, 1930.
- (8) KEILIN, D. AND E. F. HARTREE. *Proc. Roy. Soc. B.* **122**: 298, 1937.
- (9) KERLY, M. *Biochem. J.* **31**: 1544, 1937.
- (10) KHAYYAL, M. A. AND C. M. SCOTT. *J. Physiol.* **72**: 13P, 1931.
- (11) KHAYYAL, M. A. AND C. M. SCOTT. *Quart. J. Exper. Physiol.* **25**: 77, 1935.
- (12) MACLEOD, J. AND S. R. M. REYNOLDS. *Proc. Soc. Exper. Biol.* **37**: 666, 1938.
- (13) OGSTON, F. J. AND D. E. GREEN. *Biochem. J.* **29**: 1983, 1935.
- (14) PINCUS, G. *J. Exper. Zool.* **82**: 85, 1939.
- (15) PINCUS, G. AND J. BERKMAN. *This Journal* **119**: 455, 1937.
- (16) PINCUS, G. AND M. GRAUBARD. *Endocrinology*, 1940 (in press).
- (17) QUENSEL, W. AND K. WACHOLDER. *J. Physiol. Chem.* **231**: 65, 1935.
- (18) WOODWARD, G. E. AND E. G. FRY. *J. Biol. Chem.* **97**: 465, 1932.

## EFFECT OF PROGRESSIVE SYMPATHECTOMY ON HYPERTENSION PRODUCED BY INCREASED INTRACRANIAL PRESSURE

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In a previous paper (1) it was shown that total sympathectomy and complete cardiac denervation in dogs did not prevent the development of hypertension after constriction of the renal artery. At that time one of us (W. A. J.) was studying the hypertension produced by the intracisternal injection of kaolin. It was observed that this form of hypertension could be prevented by total sympathectomy (2). At approximately the same time Grimson, Wilson and Phemister (3) reported that the rise in blood pressure from increased intracranial pressure was abolished by total sympathectomy although it was unaffected by complete cardiac denervation or by pre- or postganglionic splanchnic denervation. The present experiments were undertaken to determine whether acute hypertension produced by increased intracranial pressure could be prevented by removal of a specific portion of the sympathetic nervous system rather than by total sympathectomy.

**METHODS.** A single group of dogs was used in all of the experiments reported in this paper. The response of the blood pressure and heart rate to a sudden increase of intracranial pressure was noted and then each dog was subjected to progressive sympathetic ganglionectomy. After each stage of the sympathectomy the response was again noted and compared to that observed in the same dog before operation.

Sympathectomy and cardiac denervation were performed in stages according to the technique previously described (1).

Pento-barbital anesthesia was used throughout the experiments. Twenty-five milligrams per kilogram were given intravenously. With this dosage, the corneal and cough reflexes were active.

The intracranial pressure was increased by injecting, with aseptic precautions, a sterile, warm, physiological solution of sodium chloride into the cisterna magna through a short no. 18 gauge lumbar puncture needle. The solution was injected at a constant pressure of 200 mm. of mercury.

In order to insert the needle into the cisterna, it was necessary to flex the

head upon the chest. The head was held in this position by a head holder. To ensure an adequate airway, a soft rubber tube of one-half inch bore was inserted between the vocal cords into the trachea. When apnea occurred as the result of increased intracranial pressure, respiration was maintained through this tube.

Regeneration of sympathetic nerves in experimental animals occurs extensively (3) and rapidly (4). In order to avoid the possibility of regeneration, the studies were completed within eight weeks of the commencement of the operative procedures. One week was allowed to elapse after operation before tests were made. By this time the dogs had recovered from the trauma of the operations.

The blood pressure was measured with a Hamilton (5) manometer from the femoral artery.

When saline was injected into the cisterna at a pressure of 200 mm. of mercury, the dog behaved in a characteristic manner. At first there was slight irregularity in the respiratory rhythm. Then, in ten to fifteen seconds, the dog gradually extended its legs and attempted to arch its back. Respiration ceased and the extensor rigidity changed to flaccidity. The eyeballs protruded and the edges of the tongue gradually curved over and became cyanotic. As soon as respiration ceased intratracheal insufflation at regular intervals was started in order to avoid systemic asphyxia. The pressure on the cisterna was maintained until the blood pressure had risen to over 300 mm. of mercury or for a period of one-half to two minutes. The pressure was then released and the spinal fluid allowed to drain through the needle. Spontaneous respiration was resumed after thirty to sixty seconds, depending upon the length of time that the increased pressure was maintained. By the next day the dog had entirely recovered and was ready for further procedures.

It is recognized that cerebral compression produces bradycardia through stimulation of the vagi. This bradycardia may interfere with the full development of the hypertensive reaction. In these experiments, therefore, a failure to obtain a rise in blood pressure with increased intracranial pressure was not accepted as a valid result unless the failure persisted after the vagal influence had been abolished by atropine, cervical or intrathoracic vagotomy, or by paralysis of the vagi with novocain. Under these circumstances the effectiveness of the paralysis was confirmed by absence of cardiac slowing with direct stimulation of the vagus trunk in the neck.

*A. Normal dogs.* The average resting blood pressure in eight dogs under the conditions of the experiment was 190/125. When the intracranial pressure was increased to 200 mm. of mercury, the systolic blood pressure went up to above 300 mm. of mercury. The rise of diastolic pressure was not as marked. The average increase in systolic pressure was 140 mm. and in diastolic pressure 60 mm. of mercury.

B. *Lower thoracic sympathectomy and splanchnicectomy.* After bilateral removal of the lower thoracic ganglia (T 10 - L 1), and this operation includes resection of the splanchnic nerves, the control blood pressure was slightly lower than in the normal dogs, but with the increase in intracranial pressure the rise in pressure was just as great as in the normal dogs. In a series of four dogs the average change was 130 mm. of mercury systolic and 85 mm. of mercury diastolic pressure.

C. *Upper and lower thoracic sympathectomy and splanchnicectomy.* The control level of blood pressure was not more reduced by combined bilateral upper (Stellate - T 5) and lower (T 10 - L 1) thoracic sympathectomy and splanchnicectomy than by lower thoracic sympathectomy and splanchnicectomy alone. However, when the cisternal injection was made in each of 5 experimental animals there was now no significant rise in the blood pressure.

D. *Total sympathectomy.* The resting blood pressure was 155/90 in three dogs which had recovered after total sympathectomy (Stellate - L 5). Increase in intracranial pressure brought about only a slight rise (5 to 10 mm. of mercury) in the systolic and diastolic blood pressures. Five months after completion of the sympathectomy there was still no hypertensive response to increased intracranial pressure in two of these dogs which were tested.

E. *Cardiac denervation.* When the intracranial pressure was raised in a dog in which all the cardiac nerves, both vagal and sympathetic (Stellate - T 5) had been severed within the thorax, the rise in blood pressure was just as great as that observed after upper thoracic sympathectomy alone. There was a rise in heart rate coincident with the rise in blood pressure. Figure 1 shows the results obtained in one of these experiments. A similar observation was made in three other dogs.

F. *Cardio-adrenal sympathectomy.* The dog with the denervated heart, whose reactions to increased intracranial pressure were shown in figure 1 was subjected to denervation of the left adrenal (by section of the splanchnic nerves on that side and removal of the upper lumbar sympathetic ganglia) and removal of the right adrenal gland. This operation prevented the secretion of adrenine but left one splanchnic nerve intact. After the dog had recovered from the effects of this operation, the reaction to increased intracranial pressure was again studied. The response is illustrated in the right half of the record given in figure 1. There was now no rise in blood pressure. Neither was there an increase in heart rate. The other three dogs with denervated hearts showed a similar response after removal of one and denervation of the other adrenal.

An additional experiment on cardio-adrenal sympathectomy is illustrated in figure 2. This dog had recovered from bilateral upper thoracic sympathectomy (Stellate - T 6). By this operation the cardiac sympathetic nerves had been severed. The day before the experiment, the right

adrenal gland was removed. In the acute experiment, both vagi were cut in the neck so as to produce a complete cardiac denervation. The first response to increased intracranial pressure is illustrated in the top curve in figure 2. The systolic blood pressure rose to 330 mm. of mercury and the rate of the denervated heart increased from 80 to 192 beats per minute. The left adrenal gland was then exposed at 3:57 p.m. and 1 per cent novocain injected into the medulla of the gland. Eighteen minutes later, with an identical increase of intracranial pressure, the blood pressure went up to only 200 mm. of mercury and the heart rate to 132. Thirteen minutes later, when the stimulation was repeated, a hypertensive response was again obtained together with a pronounced increase in heart rate. The gland was again paralysed by novocain at 4:35 p.m. Now, with increased intracranial pressure there was no rise in blood pressure nor change in rate

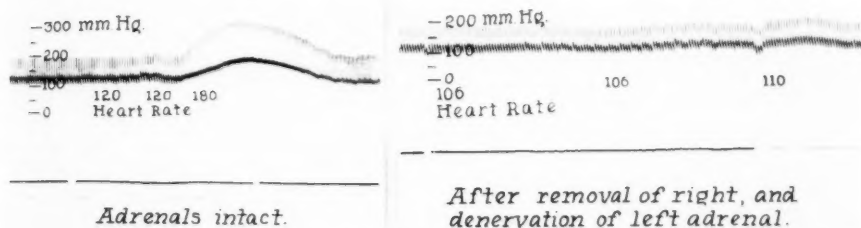


Fig. 1. The effect of increased intracranial pressure on blood pressure and rate of totally denervated heart of a dog under pento-barbital anesthesia. In this as in the following records the intracranial pressure was increased during the interval of time between interruptions of the signal line.

of the denervated heart. At 5:22 p.m., the intracranial pressure was again raised and a hypertensive response with tachycardia was obtained.

G. *Total sympathectomy except the cardiac nerves.* The experiments so far described were undertaken to ascertain how much of the sympathetic nervous system it was necessary to remove in order to abolish the hypertensive response to increased intra-cranial pressure. It was found that this pressor reaction could be abolished by denervation of the heart and the adrenal medulla but that the response persisted when the sympathetic innervation to either the heart or to the adrenal medulla was intact. It was also of interest to determine how much of the sympathetic system could be removed without preventing the hypertensive reaction. Figure 3 illustrates the record obtained in one dog. The entire right chain of paravertebral sympathetic ganglia and the left chain from the 8th thoracic to the 5th lumbar, including the splanchnic nerves, were removed in one stage. In spite of the fact that only the left upper thoracic ganglia were

present, the dog developed a full rise of blood pressure to 300 mm. of mercury when the intracranial pressure was increased just seven days after the operation. The second half of the record shows that two weeks after

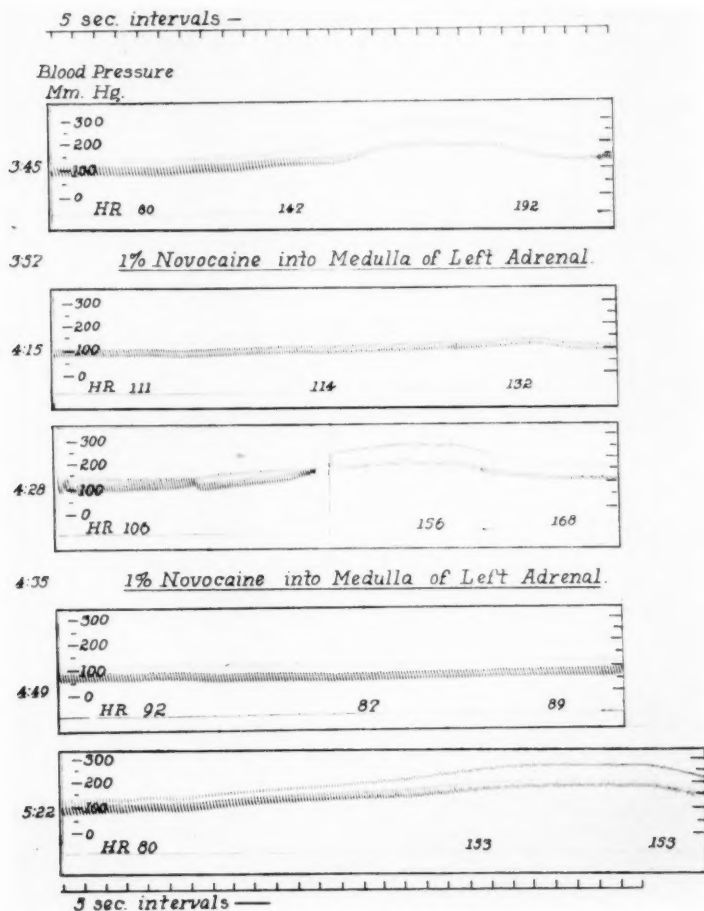


Fig. 2. The effect of increased intracranial pressure on blood pressure and rate of totally denervated heart of a dog under pento-barbital anesthesia. Right adrenal gland previously removed. The time when each record was obtained is indicated in the left hand margin. After the first and third records, 1 per cent novocain was injected into the medulla of the left adrenal gland.

completion of the sympathectomy, by removal of the left upper thoracic chain of ganglia, there was no rise in blood pressure with increased intracranial pressure.

In two other dogs it was found that the hypertensive reaction could be produced with but the upper four thoracic ganglia on either the right or the left side remaining. In one of these dogs the branches of the remaining stellate ganglion, which ran to the brachial plexus, were also resected without interfering with the rise in blood pressure. This reaction then disappeared after completion of the sympathectomy.

H. *Cardiac versus pulmonary sympathetics.* Professor Erlanger suggested (6) that the disappearance of the hypertensive response after excision of the upper thoracic sympathetic chains might have been the result of removal of the sympathetic nerves to the pulmonary vessels rather than to exclusion of sympathetic impulses from the heart. In order

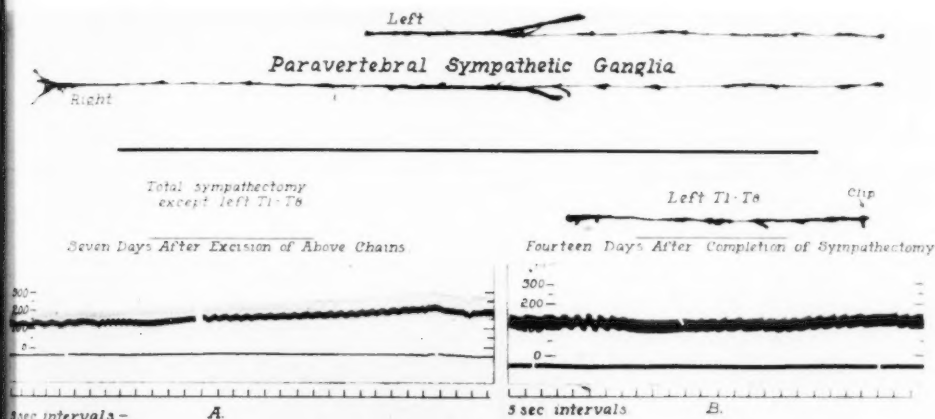


Fig. 3. The effect of increased intracranial pressure on blood pressure of a dog: A, seven days after subtotal sympathectomy. B, fourteen days after completion of the sympathectomy. The ganglionated sympathetic chains removed at operations are shown above the blood pressure records.

to answer this question the left lung was excised by the technique of Willy Meyer (7). The dog was then subjected to bilateral lower (T 9 - T 13) thoracic and right upper (Stellate - T 5) thoracic sympathectomy. The reaction to increased intracranial pressure was then tested before and again after removal of the left upper thoracic sympathetic ganglia. Before the final operation there was a full hypertensive response to increased intracranial pressure. After removal of the upper thoracic sympathetic ganglia on the side from which the lung had previously been removed, there was no significant increase in blood pressure.

**Discussion.** It is recognized that the physiological reactions produced by injection of fluid into the subarachnoid space result from compression of the medulla, with resultant anemia of the autonomic centers (8).

Anemia of the medulla has been produced experimentally in various other ways: introduction of a solid body into the calvarium, injection of particulate matter into the carotid arteries, or occlusion of the carotid and vertebral arteries. Since the physiological reactions are the same in spite of different methods of producing medullary anemia, no distinction will be made in this discussion between the various ways of bringing about this condition.

Since the fundamental work of Naunyn and Schreiber (9) it has been recognized that cerebral anemia brings about an increase in blood pressure. This increase in blood pressure is prevented by section of the spinal cord in the cervical region (10). Cushing (8), who studied the reaction closely, found that the rise of blood pressure bore a quantitative relationship to the increase in intracranial pressure. He also observed that the reaction disappeared after division of the spinal cord in the high cervical region or after injecting cocaine into the medulla oblongata. Since he saw constriction of the arteries of the splanchnic area with cerebral compression, he assumed that the attendant rise in blood pressure was the result of this constriction. Stewart, Guthrie, Burns and Pike (11) also observed the constriction of the splanchnic arteries with cerebral anemia and correlated this reaction with the rise in blood pressure. It seems to be well established that anemia of the medulla brings about hypertension through activity of the autonomic centers transmitted through the spinal cord. With the anemia there is also vasoconstriction of the splanchnic area.

Since the rise in blood pressure from medullary anemia is mediated through the spinal cord and is associated with splanchnic vasoconstriction and since it disappears after section of the spinal cord in the cervical region, a procedure which is known to abolish splanchnic vasoconstriction, it is natural to assume that the rise in blood pressure is the result of this vascular contraction.

The part played by medulli-adrenal secretion in the rise in blood pressure from cerebral anemia was suggested by Roger (12), who observed that the rise in blood pressure was not as great nor as sustained after removal of the adrenal glands. Tournade (13) demonstrated clearly by crossed circulation experiments that an increase of medulli-adrenal secretion took place under these circumstances.

Further analysis of the mechanism of the rise of blood pressure from cerebral anemia was made by Yates (14). She found that the "anemic rise" could not be prevented by section of the spinal cord even as high as the 1st thoracic level or by removal of the stellate ganglia. However, if the spinal cord was cut at the third or even first thoracic level, the "anemic rise" did disappear when the stellate ganglia were also removed. She was unable to explain this reaction but suggested that the extrinsic cardiac

nerves were of significance after the vasomotor system had been excluded by high section of the spinal cord.

Winkin (15) confirmed these observations but explained them on the supposition that splanchnic fibers left the spinal cord to run in the sympathetic chains from as high up as the 2nd and 3rd roots, even though she admitted that the preponderant evidence was to the contrary. She concluded that "the nerves of the heart are not essential either for the activation or for the persistence of the characteristic pressor phenomena of the 'anemic rise'," and that "The activation and maintenance of the vascular response under cerebral occlusion is controlled essentially by the splanchnic nerves." Although she recognized the fact that the adrenal glands were involved in the reaction she did not consider their secondary action upon the heart.

The observations which we have made are quite in harmony with these experiments reported in the literature. Our results also confirm those of Grimson, Wilson and Phemister (3) to the extent that neither complete cardiac denervation nor splanchnic sympathectomy alone sufficed to prevent the development of hypertension from cerebral compression but that the rise in blood pressure could be prevented by total sympathectomy. However, they did not investigate the rôle of the adrenal medulla in the pressor response.

When the intracranial pressure was increased in the dog with the denervated heart, as figure 1 shows, there was an increase in blood pressure. The increase in heart rate coincided with the pressor response. This increase in rate must have been due to the action of adrenaline since the heart was denervated.

As Tournade pointed out, cerebral anemia stimulates adrenal secretion. It also causes splanchnic vasoconstriction (8, 11). These two phenomena probably occur simultaneously since the anatomic arrangement of the sympathetic nervous system is such as to facilitate a diffuse discharge of sympathetic impulses (16). It accordingly seemed likely that at the time that the secretion of adrenaline took place, in the experiment illustrated in figure 1, there was concomitant splanchnic vasoconstriction. The secretion of adrenaline must have occurred some time before its effect upon the heart could have been observed, i.e., the time required for the blood from the adrenal gland to reach the heart. Inspection of the record shows no significant rise in blood pressure in the time immediately preceding the increase in heart rate, and yet during this time it is likely that reflex splanchnic vasoconstriction was taking place. The possibility was thus presented that splanchnic vasoconstriction occurred without hypertension until the adrenaline reached the heart. Subsequent experiments (figs. 1 and 2) in which the medulli-adrenal secretion was prevented from reaching

the heart, without interference with the splanchnic outflow of vasoconstrictor impulses, confirmed our supposition. When both neural and humoral sympathetic impulses were excluded from the heart, the rise in blood pressure from cerebral compression was prevented, even though no effort was made to prevent splanchnic vasoconstriction.

The observation that the hypertensive response persisted in spite of removal of all but the upper thoracic sympathetic ganglia on one side (fig. 3) agrees with the observations of Heymans and Bouckaert (17), who showed in a dog that the rise in blood pressure after denervation of the carotid sinus and section of the depressor nerves persisted until removal of the last few sympathetic ganglia in the left upper thorax. Since we showed in another dog that removal of the left upper thoracic sympathetic ganglia was effective in abolishing a hypertensive response even when the lung on that side had previously been removed, it seems probable that the significant factor in this operation was the sympathectomy of the heart.

**SUMMARY.** The effect of progressive sympathectomy upon the rise in blood pressure induced by increased intracranial pressure was studied in dogs under pento-barbital anesthesia. The experiments were performed under aseptic precautions and the same group of dogs was used throughout.

Bilateral lower thoracic sympathectomy (T 10 - L 1) including splanchnicectomy did not prevent the development of hypertension from increased intracranial pressure.

Complete cardiac denervation (Stellate - T 6, and vagus fibers) did not interfere with the full development of the pressor response.

The increase in blood pressure from cerebral compression was prevented by bilateral upper (Stellate - T 5) and lower (T 10 - L 1) thoracic sympathectomy. It was also abolished by total sympathectomy.

Evidence is presented to indicate that the lower thoracic sympathetic impulses facilitate the development of the hypertensive reaction through the medium of adrenal secretion which acts upon the heart. A rise in blood pressure from cerebral compression can be obtained in a dog with sympathetic cardiac denervation, only if medulli-adrenal secretion is present.

Extensive sympathectomy which leaves intact only the upper thoracic sympathetic ganglia (Stellate - T 4) on one side, does not prevent the development of a rise in blood pressure under the experimental conditions. Evidence is presented that the hypertensive reaction is dependent upon the activity of the cardiac rather than upon the pulmonary sympathetics.

#### CONCLUSION

Sympathetic cardiac innervation, neural or humoral, is necessary for the development in anesthetized dogs of the rise in blood pressure produced by increased intracranial pressure.

## REFERENCES

- (1) FREEMAN, N. E. AND I. H. PAGE. *Am. Heart J.* **14**: 405, 1937.
- (2) PAGE, I. H. *Bull. New York Acad. Med.* **13**: 645, 1937.
- (3) GRIMSON, K. S., H. WILSON AND D. B. PHEMISTER. *Ann. Surg.* **106**: 801, 1937.
- (4) SIMEONE, F. A. *This Journal* **120**: 466, 1937.
- (5) HAMILTON, W. F., G. BREWER AND I. BRATMAN. *This Journal* **107**: 427, 1934.
- (6) ERLANGER, J. Personal communication.
- (7) MEYER, W. *J. A. M. A.* **53**: 1978, 1909.
- (8) CUSHING, H. *Johns Hopkins Hosp. Bull.* **12**: 290, 1901.
- (9) NAUNYN, B. AND J. SCHREIBER. *Arch. f. exper. Path. u. Pharmakol.* **14**: 1, 1881.
- (10) NAWALICHIN, J. *Centralblt. f. Med. Wiss.* **8**: 483, 1870.
- (11) STEWART, G. N., C. C. GUTHRIE, R. L. BURNS AND F. H. PIKE. *J. Exper. Med.* **8**: 289, 1906.
- (12) ROGER, H. *Arch. de Med. exp. et d'Anat. Path.* **27**: 591, 1916-17.
- (13) TOURNADE, A. *J. Med. Franc.* **14**: 206, 1925.
- (14) YATES, A. B. *This Journal* **57**: 68, 1921.
- (15) WINKIN, C. S. *This Journal* **60**: 1, 1922.
- (16) CANNON, W. B. *Lancet* **1**: 1109, 1930.
- (17) HEYMANS, C. AND J. J. BOUCKAERT. *Compt. rend. Soc. Biol.* **120**: 82, 1935.

## THE PRESSOR EFFECTS OF HOMOLOGOUS AND HETEROLOGOUS INJECTIONS OF HEATED KIDNEY EXTRACTS

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It has been shown repeatedly that certain extracts of normal kidney tissue contain an apparently unique pressor substance which has been called "renin" (1-10). Although kidney extracts from various animals are said to differ with respect to their content of renin, no systematic study of specific variation in this respect has been made. Moreover, the content of renin has been determined in many instances by injecting into one species the crude or refined extracts of kidney tissue obtained from another species. Because of earlier statements that the results obtained by such heterologous injections are totally undependable (3, 4) and because heterologous injections are obligatory for assays of human kidney extracts, it seemed advisable to re-examine the question.

The observations described in this paper were therefore designed to *a*, compare the pressor activities of extracts from the normal kidneys of several species, using a uniform extraction method; *b*, observe whether the selected species respond equally to their own and to heterologous extracts, and *c*, determine under what conditions heterologous injections can be used justifiably for assay purposes.

**METHODS AND MATERIAL.** Simple saline extracts of kidney tissue are frequently depressor and lethal even when injected into the same species (5, 6). It has been shown, however, that heating such extracts to 55°C. for twenty minutes, with subsequent filtration, yields water-clear solutions which, when injected into unanesthetized rabbits, elevate the blood pressure in a characteristic manner without diminishing peripheral blood flow appreciably (6). This procedure was used throughout the following studies because these heated extracts are as a rule non-toxic and exhibit neither serious depressor nor significant tyramine-like action (6).

Kidneys were removed from normal rats, guinea pigs, rabbits and dogs after they had been killed by concussion and thoroughly bled by cutting

<sup>1</sup> The expenses of this investigation were defrayed in large part by a grant from the Commonwealth Fund.

the vessels of the neck. Human kidneys were obtained at autopsy from three to fifteen hours after death, grossly diseased kidneys being excluded. After the capsule and hilar tissues were removed, the entire parenchyma was cut into small fragments.

Enough distilled water was added to the cut tissue to make a 20 per cent mixture which was then ground in a water-cooled colloid mill for five minutes at a temperature not above 35°C. The crude emulsion was immediately placed in a water bath at 56°C., allowed to warm gradually to between 55° and 56°C., and kept at that temperature for twenty minutes. During this heating from 25 to 50 per cent of the protein was precipitated as a coagulum. The extract was then filtered through two layers of no. 1 Whatman paper in funnels heated to 55°C. The initial filtrate, which was more or less cloudy, was poured back into the funnels until a clear, yellowish (reddish for human kidneys) fluid was obtained. The freshly prepared extracts were placed in ampules holding up to 25 cc., and dried in vacuo from the frozen state by the Cryochem process described by Flossdorf and Mudd (11). After processing was complete the ampules were sealed under vacuum. This dry powder could be kept in the refrigerator for at least several weeks without perceptible deterioration.

Total nitrogen and non-protein nitrogen were determined by micro-Kjeldahl digestion followed by direct Nesslerization as described by Koch and McMeekin (12) using a photoelectric colorimeter. The concentrations of albumin and globulin were determined after adding solid sodium sulphate to the extract in amounts sufficient to yield a final concentration of 1.5 M. of sulphate (13).

Shortly before an injection was to be made, the ampule was opened and 0.9 per cent NaCl was added to the amount required to make 60, 50 or 10 per cent extracts; each cubic centimeter of the final solution then contained the solid extractives of 0.6, 0.5 or 0.1 gram of kidney tissue, respectively. The redissolved extracts were clarified by centrifuging and warmed to 39°C. before injection. For control injections potent extracts were inactivated by heating to 80°C. for 5 minutes, which destroys or precipitates renin (1, 6). These inactivated extracts were filtered, centrifuged, warmed to 39°C. and injected as control solutions to reveal effects that might be due to extraneous substances and salts.

In preliminary experiments<sup>2</sup> unanesthetized rabbits were used according to the method previously described (6). They were warmed to abolish vasoconstrictor tone, while systolic blood pressure, pulse rate and the amplitude of pulsation in the auricular artery were measured by means of a recording oscillogram. Changes in blood flow through the skin were followed by measuring skin temperature thermoelectrically. Twenty cubic

<sup>2</sup> Chiefly observations 1 and 2, page 674 and 675.

centimeters of 10 per cent extract were injected slowly at the rate of 1.5 to 1.6 cc. per minute in order to allow for the delay with which changes in skin temperature follow changes in peripheral blood flow.

For testing systematically the comparative pressor effects of homologous and heterologous heated kidney extracts,<sup>3</sup> rats, guinea pigs, rabbits and dogs were anesthetized lightly with nembutal intraperitoneally in doses of 30 to 40 mgm. per kilo body weight, supplemented if necessary by smaller amounts subcutaneously. To avoid the effects of deep anesthesia, dosage was adjusted so that corneal reflexes remained quite active and the animals moved when stimulated.

Blood pressures in these animals were measured by means of the Hamilton-manometer, using a gauge-20 needle to enter the exposed femoral artery which had previously been bathed locally with 1 per cent novocaine in order to produce maximal dilatation. For rabbits, a femoral cannula and simple Hürthle manometer were used. To avoid disturbing the animal all injections were made through a gauge-24 needle in the form of a T-cannula which had been tied into a superficial vein (femoral or auricular) at the beginning of the experiment.

Dosage was adjusted so that each animal received in one minute 1.0 cc. of extract per kilo body weight; each cubic centimeter of extract contained the equivalent of 0.5 to 0.7 gram kidney tissue. Typical doses were:—rats, 0.25 cc. of 50 per cent extract; guinea pigs, 0.5 cc. of 50 per cent extract; rabbits, 2.0 to 2.5 cc. of 50 or 60 per cent extract; and dogs, 5.0 to 5.5 cc. of 50 per cent extract. Control injections, in similar volume of homologous serum, 0.9 per cent NaCl, and kidney extracts heated to 80°C. did not affect blood pressure significantly.

**OBSERVATIONS.** 1. *Effects of rapidly repeated injections of rabbit kidney extract into rabbits (tachyphylaxis).* In order to determine whether repeated injections at short intervals were permissible for assay purposes, portions of the same extract of rabbit kidney were injected at intervals of thirty minutes into both anesthetized and unanesthetized rabbits. A typical result is shown in figure 1. With or without anesthesia the first injection of a potent extract elevated blood pressure markedly, but later doses became less and less effective. In control experiments, to exclude possible effect of prolonged body warming or anesthesia, the first two injections consisted of 0.9 per cent NaCl solution, only the third injection consisting of rabbit kidney extract. In each instance a conspicuous rise of blood pressure followed the last injection, showing that it was the prior injection of kidney extract, and not the anesthesia or body warming, which was responsible for the diminished effect of the second and third injections. In the rabbit, under the conditions of these experiments, it appears that rapidly repeated injections are undesirable when the pressor activity

<sup>3</sup> Observation 3, page 677.

of kidney extracts is being determined quantitatively. In agreement with others (1, 5) we found that animals began to regain their responsiveness to potent extracts in one or two hours.

2. *Effects of homologous and heterologous injections of heated kidney extracts into rabbits at intervals of seven days or more (anaphylaxis).* If a given rabbit kidney extract was injected at intervals of one week or more into the same rabbit, the successive pressor responses were approximately equal, though slight increase in sensitivity of the injected animal could not be excluded definitely (fig. 2). After each injection blood pressure rose in the usual manner without changing either skin temperature or amplitude of arterial pulsation.

Entirely different effects appeared when human kidney extracts were injected into the same unanesthetized rabbit at intervals of one week or

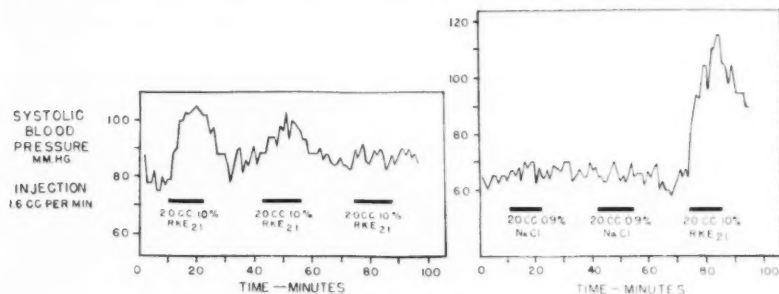


Fig. 1. *Left.* Showing diminishing effect obtained by repeated injection of the same rabbit kidney extract into warmed unanesthetized rabbits.

*Right.* Control experiment showing that sustained reactivity to rabbit kidney extract despite prolonged body warming (rectal temperature 41.0-41.5°C.).

more (fig. 3). In this experiment the first dose of human kidney extract produced no significant rise in systolic pressure and did not change pulse amplitude, skin temperature or heart rate. Approximately two weeks later another sample of this same extract produced in this rabbit a sharp drop in systolic blood pressure, blanched the ear, obliterated the pulsations in the central artery of the ear, and conspicuously reduced skin temperature. In the rabbit, constriction of the vessels of the ear has been described as a characteristic part of the anaphylactic response following the second injection of horse serum (14). We have observed similar reactions in unanesthetized rabbits after appropriately spaced injections of kidney extracts of rat, dog, and guinea pig, and after injection of horse and human serum.

In order to test for possible deterioration of the extract itself, the same material which produced the anaphylactic response in the first animal was

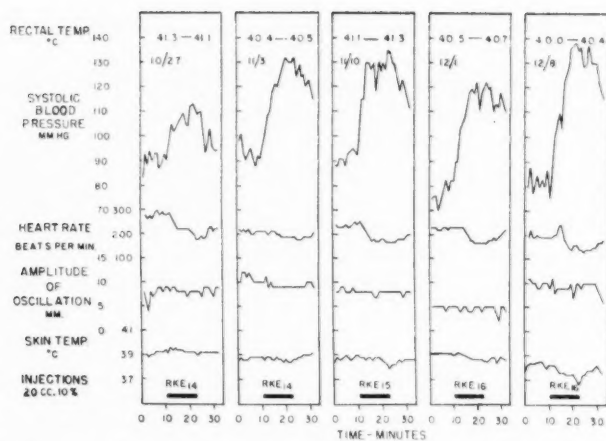


Fig. 2. Showing effect of injecting heated rabbit kidney extract (corresponding to 2.0 grams kidney tissue in 12 min.) into the same unanesthetized rabbit at intervals of 7 days or more.

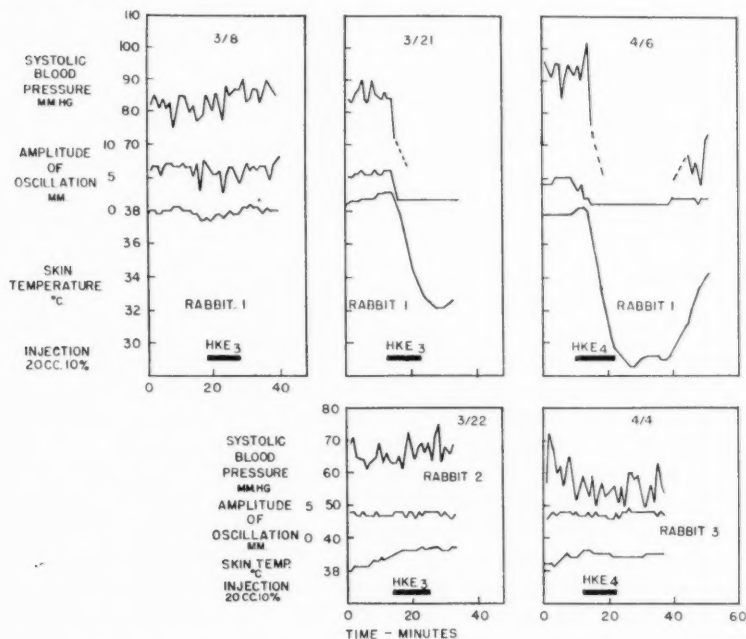


Fig. 3. Effect of initial and later injections of human kidney extract into the unanesthetized rabbit.

*Upper.* Rabbit 1, sensitized by the initial injection, exhibited vascular changes suggesting anaphylaxis on the second and third injections.

*Lower.* The same extract injected into normal animals (2 and 3) did not change blood pressure, pulse amplitude or skin temperature significantly.

injected into a second animal not previously injected (lower left, fig. 3). In this control experiment the extract was as inert as in the initial injection of the first animal. The results of a third injection and its control are also shown in the right-hand portion of figure 3. If injections into sensitized rabbits are performed slowly and if they are terminated as soon as the ear blanches slightly, the animal survives. On the other hand, rapid injection into sensitized animals is often followed by respiratory distress and death. It was found that the blood serum of sensitized rabbits contains specific precipitins after the repeated injection of a foreign kidney extract but no precipitins have been identified after the repeated injection of rabbit kidney extract.

Therefore, in the rabbit, while heated rabbit kidney extracts are benign, foreign kidney extracts, if injected at intervals of one to two weeks, diminish blood pressure conspicuously and produce symptoms resembling those of anaphylactic shock. It follows that the initial injection is the only one which can be used safely to identify pressor activity of heterologous extracts. It is conceivable, however, that purified renin might not produce anaphylaxis and that animals which are less easily sensitized (e.g., dogs and rats) might not show anaphylaxis even to crude extracts.

3. *Comparative effects of single homologous and heterologous injections of kidney extracts of rats, guinea pigs, rabbits, dogs and man.* The dried samples of kidney extracts, after being dissolved in salt solution, were first injected into unanesthetized rabbits in order to test their relative toxicity by their effects on skin temperature, arterial pulsation, and pulse rate. The results of these determinations are shown in table 1. While differing conspicuously in their pressor activity, the extracts did not reduce skin temperature excessively and they were not toxic. The injected animals survived until sacrificed some weeks later. When tested in these unanesthetized rabbits under standard conditions, the heated kidney extracts of dog and man were least active, those of the rabbit most active, and those of the rat and guinea pig intermediate.

In the comparative studies (table 2) the test animals, excepting dogs, received only one dose of kidney extract to avoid the errors produced by tachyphylaxis and anaphylaxis. In the dog, because of expense and the unlikelihood of routine use for assay injections, several doses were given to the same animal at intervals of one hour or more, with a total series of four or five injections per experiment. This allowed considerable time for recovery but, nevertheless, in successive experiments the injections were rotated so that a fair average result might be obtained.

The effects produced by kidney extracts injected rapidly in standard dosage into animals lightly anesthetized with nembutal, varied according to the source of extract and according to the species of the recipient animal, as shown in table 2 in which only changes of systolic blood pressure

are recorded because mean and diastolic pressures changed roughly in proportion. In some instances (about 15 per cent of experiments) a preliminary fall of blood pressure was produced, but this depression lasted thirty seconds or less and was minor in grade. In two animals, however, a more prolonged reduction in blood pressure was observed; this may have been due to the anesthetic or to individual abnormality, since the

TABLE 1  
*Effects of heated kidney extracts on blood pressure and skin temperature of the unanesthetized rabbit\**

| SOURCE OF KIDNEY EXTRACT | CHANGE OF BLOOD PRESSURE | CHANGE OF HEART RATE  | CHANGE OF SKIN TEMPERATURE | TIME OF MAXIMUM BLOOD PRESSURE |
|--------------------------|--------------------------|-----------------------|----------------------------|--------------------------------|
|                          | <i>mm. Hg</i>            | <i>beats per min.</i> | <i>°C.</i>                 | <i>min.</i>                    |
| Rat .....                | +10                      | -10                   | 0                          |                                |
|                          | +5                       | +30                   | -0.3                       |                                |
|                          | +5                       | +20                   | 0                          |                                |
| Guinea pig .....         | +10                      | 0                     | 0                          |                                |
|                          | +5                       | +20                   | -0.4                       |                                |
|                          | +5                       | -30                   | +0.5                       |                                |
| Rabbit .....             | +40                      | -80                   | -0.6                       | 3                              |
|                          | +35                      | -150                  | -0.6                       | 2                              |
|                          | +40                      | -60                   | -0.1                       | 3                              |
|                          | +45                      | -200                  | -0.1                       | 2                              |
| Dog .....                | +10                      | 0                     | -0.2                       |                                |
|                          | 0                        | +10                   | +0.3                       |                                |
|                          | 0                        | 0                     | -0.8                       |                                |
| Man .....                | 0                        | 0                     | -0.3                       |                                |
|                          | 0                        | +10                   | +0.4                       |                                |
|                          | +5                       | +20                   | 0                          |                                |

\* Dose: Each animal received 20 cc. of 10 per cent extract, injected at the rate of 1.6 cc. per minute.

same extract in other animals elevated blood pressure slightly. One of these animals had grossly abnormal kidneys on postmortem examination.

Typically, for homologous and heterologous injections alike, blood pressure rose slowly, reaching a maximum in two to four minutes. Changes in heart rate ranged from conspicuous slowing to slight increase of rate. Breathing often became deeper and more rapid temporarily, whether or not blood pressure was elevated.

Table 2, which summarizes the results, indicates that rabbit kidney extracts were very active in all recipients, the average change in blood pressure with similar dosage being 43 mm. Hg. Kidney extracts from the

guinea pig and rat were considerably less active in general, producing an average rise of only 21 mm. Hg. The extracts from dog and man were least active. Homologous injections were not uniformly more pressor than heterologous injections. The four recipient species differed, however, in their responsiveness to this group of extracts in that greater elevations

TABLE 2

*Effects of heterologous injections of heated kidney extracts on systolic blood pressure (light nembutal anesthesia)\**

| SOURCE OF EXTRACT | RECIPIENT ANIMAL    |                     |                     |                     | AVERAGE RISE OF BLOOD PRESSURE |
|-------------------|---------------------|---------------------|---------------------|---------------------|--------------------------------|
|                   | Rat                 | Guinea pig          | Rabbit              | Dog                 |                                |
|                   | <i>mm. Hg</i>       | <i>mm. Hg</i>       | <i>mm. Hg</i>       | <i>mm. Hg</i>       |                                |
| Rat               | +24<br>(+10 to +50) | +36<br>(+10 to +55) | +6<br>(0 to +15)    | +17<br>(0 to +40)   | +21                            |
| Guinea pig        | +26<br>(+10 to +55) | +48<br>(+30 to +65) | +6†<br>(-20 to +12) | +3<br>(0 to +10)    | +21                            |
| Rabbit            | +52<br>(+20 to +80) | +38<br>(+15 to +55) | +40<br>(+35 to +45) | +42<br>(-10 to +90) | +43                            |
| Dog               | +8<br>(0 to +10)    | +15<br>(+10 to +25) | +3<br>(0 to +15)    | +7<br>(0 to +22)    | +8                             |
| Man               | +4<br>(0 to +15)    | 0<br>(-10 to +5)    | +2<br>(0 to +10)    | +8<br>(0 to +15)    | +5                             |
| Average . . . .   | +23                 | +27                 | +12                 | +15                 |                                |

\* Figures refer to 3 or more animals; range of changes shown in parentheses.

Dose: Each animal received in one minute 1.0 cc. of extract per kilo of body weight; each cubic centimeter of extract contained the equivalent of 0.5 to 0.7 gram kidney tissue.

† Depressor response excluded from average since recipient animal at autopsy had abnormal kidneys. Other injections were slightly pressor.

of blood pressure were observed in the guinea pig and rat than in the rabbit and dog.

Because renin can be salted out by half saturation with ammonium sulphate, it appears likely that it is a globulin-like substance or is closely associated with globulin (1, 2, 5, 6). The concentrations of albumin and globulin were determined in all of the heated extracts to ascertain whether the pressor activity of the extract was directly related to the total amount of globulin present. Table 3 shows that active extracts (e.g., rabbit, guinea pig and rat) contained no more globulin than did the relatively

inactive extracts (dog and man). Contamination of extracts by blood plasma was not excluded entirely; the animals were bled thoroughly but the renal vessels were not washed out.

DISCUSSION. Assays of the pressor activity of kidney extracts have so far been inconsistent, probably because of the number of variables involved. The opposing results obtained by different investigators are, however, being explained gradually as these variables are identified. Thus simple saline extracts of normal or abnormal kidneys are lethal (5, 6), unpredictable as to action (3, 4), or yield mixed depressor or pressor effects (7, 8). Toxicity and depressor activity can be reduced by suitable heating (6), by extraction with alcohol (1, 5), or by precipitation with suitable concentrations of ammonium sulphate (2, 5, 6). In these experiments heating was chosen because of its simplicity and because the rabbit kidney extracts thus prepared produced uniformly a rise of blood pressure

TABLE 3  
*Protein content of heated kidney extracts, 10 per cent*

|                 | NUMBER OF EXTRACTS | ALBUMIN              | GLOBULIN             |
|-----------------|--------------------|----------------------|----------------------|
|                 |                    | <i>gram per cent</i> | <i>gram per cent</i> |
| Rat.....        | 3                  | 0.10                 | 0.12                 |
| Guinea pig..... | 3                  | 0.10                 | 0.11                 |
| Rabbit.....     | 5                  | 0.09                 | 0.16                 |
| Dog.....        | 3                  | 0.07                 | 0.15                 |
| Man.....        | 8                  | 0.13                 | 0.14                 |

without decreasing peripheral blood flow,—a relationship characteristic of the hypertensive state in man.

Anesthesia, particularly by ether or urethane, may reverse a pressor to a depressor response (5). While it would have been preferable to avoid anesthesia altogether, it was impossible to do so in this group of animals. According to Pickering (5) small doses of nembutal interfere least with the reaction to renin. In our own studies pressor responses were approximately equal in rabbits without anesthesia and with light nembutal or paraldehyde anesthesia.

The results indicate that the same procedure applied to kidney tissue from several species, produces extracts of different potency. Though these results suggest that the content of renin differs according to species, it cannot safely be concluded that this is the case until similar observations have been made with other extraction methods. It is likely that such species differences do exist since Friedman et al. (9) found with extraction by alcohol that renin is more abundant in the kidney tissue of rabbits or cats than in the kidney tissue of sheep, bulls, dogs, and pigs. Other

possible causes for these variations are not excluded but, in any case, differences in normal kidney tissue according to species must be added to the variables already mentioned.

Diminishing effects on blood pressure from quickly repeated injections of a potent kidney extract (tachyphylaxis) have been observed also by others (1, 2, 9, 10). Pickering and Prinzmetal (5) state that injections for assay purposes can be repeated safely at intervals of an hour or more if blood pressure has returned to normal. Nevertheless for accurate assay, in the rabbit at least, it seems preferable to allow twenty-four hours to elapse, or even to use single injections, in order to avoid persisting effects from previous doses of kidney extract.

When heterologous injections are necessary, as in assays of extracts prepared from human kidneys, anaphylaxis may become a complicating factor when the rabbit is used and if the interval between injections is too long. Whether anaphylaxis is due to the foreign renin itself or to other proteins cannot be determined until purified renin is available. Partially purified hog renin has been injected repeatedly into dogs without evidence of anaphylaxis (15); this may be due to brief intervals between injections, to the method of extraction, or to the difficulty with which anaphylaxis is induced in the dog. Nevertheless in assays the danger of anaphylaxis is an additional reason for using single injections.

Species differences in response to kidney extracts have been suggested by Harrison et al. (10). The results of our comparative study agree in indicating that with the extracts used, guinea pigs and rats exhibit larger changes in blood pressure than do rabbits and dogs under similar circumstances. Although Pearce (3) and Miller (4) concluded that heterologous injections cannot be relied upon as a measure of pressor activity, it would seem that the pressor activity of properly prepared extracts can be tested adequately by heterologous injections providing anaphylaxis is avoided and if allowance is made for differences in the reactivity of the recipient animals.

#### SUMMARY

Rapidly repeated injections of rabbit kidney extracts into intact animals, with or without anesthesia, lead to a diminishing response (tachyphylaxis).

Rabbits receiving rabbit kidney extracts at intervals ranging from 24 hours to 7 or 10 days exhibit no significant change of resting blood pressure or conspicuous change of sensitivity to renin.

When rabbits have received an injection of similarly prepared extract from another species, subsequent injection of that extract, after a suitable interval, produces respiratory distress, a sharp drop in blood pressure, blanching of the ear, arteriolar constriction in the ear, and death if the

injection is not stopped immediately. The general picture is that of anaphylactic shock.

In performing assays of kidney extracts it seems advisable to use single injections in order to avoid both tachyphylaxis and anaphylaxis.

Potent kidney extracts raise blood pressure in the species of origin and in other species as well. Extracts of normal rabbits' kidneys are most actively pressor, those from rats and guinea pigs somewhat less active, and those from man and dog least active, when prepared by heating to 55°C. for 20 minutes.

The reactivity of these species varies, however, in that with a given group of extracts, the rat and guinea pig show more conspicuous elevation of blood pressure than do the dog and rabbit after injection of equivalent doses.

#### REFERENCES

- (1) TIGERSTEDT, R. AND P. G. BERGMAN. *Skand. Arch. f. Physiol.* **8**: 223, 1898.
- (2) BINGEL, A. AND E. STRAUSS. *Deutsch. Arch. f. klin. Med.* **96**: 476, 1909.
- (3) PEARCE, R. M. *J. Exper. Med.* **11**: 430, 1909.
- (4) MILLER, J. L. AND E. M. MILLER. *J. Physiol.* **43**: 242, 1911.
- (5) PICKERING, G. W. AND M. PRINZMETAL. *Clin. Sci.* **3**: 211, 1938.
- (6) LANDIS, E. M., H. MONTGOMERY AND D. SPARKMAN. *J. Clin. Investigation* **17**: 189, 1938.
- (7) HARRISON, T. R., A. BLALOCK AND M. F. MASON. *Proc. Soc. Exper. Biol. and Med.* **35**: 39, 1937.
- (8) PRINZMETAL, M. AND B. FRIEDMAN. *Proc. Soc. Exper. Biol. and Med.* **35**: 122, 1937.
- (9) FRIEDMAN, B., D. I. ABRAMSON AND W. MARX. *This Journal* **124**: 285, 1938.
- (10) HARRISON, T. R., A. BLALOCK, M. F. MASON AND J. R. WILLIAMS. *Arch. Int. Med.* **60**: 1058, 1937.
- (11) FLOSDORF, E. W. AND S. MUDD. *J. Immunol.* **34**: 469, 1938.
- (12) KOCH, F. C. AND T. L. McMECKIN. *J. Am. Chem. Soc.* **46**: 2066, 1924.
- (13) HOWE, P. E. *J. Biol. Chem.* **49**: 93, 1921.
- (14) ABELL, R. G. AND H. P. SCHENCK. *J. Immunol.* **34**: 195, 1938.
- (15) LEITER, L. AND L. EICHELBERGER. *J. Clin. Investigation* **18**: 477, 1939.

## CHANGES IN THE BRAIN RESULTING FROM DEPLETION OF EXTRACELLULAR ELECTROLYTES<sup>1</sup>

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In previous studies (1, 2) it has been shown that, in general, the osmotic adjustment to loss of extracellular electrolyte is brought about chiefly by changes in the distribution of body water. In brief, this probably represents a shift of water from depots of extracellular water such as the skin and blood plasma to the body cells. Whether all tissues exhibit this redistribution of water or whether some tissues adjust by other mechanisms than that cited above is a matter for further investigation. The purpose of the present communication is to describe changes in the distribution of water and electrolytes in the brain resulting from depletion of extracellular electrolytes.

**PROCEDURE.** Cats, anesthetized with sodium pentobarbital, were used as the experimental animals. The method employed for producing depletion of extracellular electrolyte was that described previously (1). Briefly, this consisted of injecting a 5 per cent solution of glucose intraperitoneally, allowing 4 to 5 hours to elapse for equilibrium to take place and then removing the peritoneal fluid which now contained considerable amounts of sodium and chloride. The quantity of glucose solution injected varied from 50 to 125 cc. per kilogram of body weight, depending on the degree of depletion desired. In order to obtain a concentration of chloride in the serum less than about 85 milliequivalents per liter, it was usually necessary to repeat the procedure on two successive days using approximately 100 cc. of glucose solution per kilogram on each occasion. In some of the more severely depleted animals, isotonic sodium sulfate solution was injected intraperitoneally immediately following the removal of the equilibrated glucose solution. Twenty to twenty-four hours after the last removal of fluid from the peritoneal cavity, the animals were anesthetized and a sample of blood removed by cardiac puncture. The

<sup>1</sup> This study was aided by a grant from The Child Neurology Research Fund (Friedsam Foundation) and the Fluid Research Fund, Yale University School of Medicine.

animals were then killed by severing the great vessels at the base of the heart. The brain was exposed by stripping the dura and both hemispheres were removed for analysis. Before being placed in weighed containers, as much as possible of the cerebrospinal fluid on the surface and in the ventricles was removed. This procedure was different from that used in previous studies (3, 4, 5), where no effort was made to remove the cerebrospinal fluid, and explains the differences in concentration of water, chloride and sodium found in the brains of those animals with essentially normal concentrations of sodium and chloride in the serum. The chemical methods have been previously described (4).

**RESULTS.** The data being presented were obtained from the analyses of the brains of 38 animals. Of these, 11 were essentially normal; the remaining 27 animals were depleted of extracellular electrolyte to varying degrees, as previously described. For the sake of simplicity in the presen-

TABLE 1

| GROUP | NUMBER | SERUM (PER L.)   |           |           | BRAIN (PER KG. FAT-FREE TISSUE) |             |             |              |           |             |
|-------|--------|------------------|-----------|-----------|---------------------------------|-------------|-------------|--------------|-----------|-------------|
|       |        | H <sub>2</sub> O | Cl        | Na        | H <sub>2</sub> O                | Cl          | Na          | Non-lipoid N | K         | K/N         |
|       |        | cc.              | m.eq.     | m.eq.     | cc.                             | m.eq.       | m.eq.       | grams        | m.eq.     |             |
| 1     | 11     | 941 ± 3.0        | 117 ± 1.2 | 150 ± 1.2 | 842 ± 3.5                       | 38.4 ± 1.1  | 57.6 ± 0.80 | 17.0 ± 0.10  | 103 ± 1.0 | 6.05 ± 0.05 |
| 2     | 5      | 926 ± 3.0        | 107 ± 1.7 | 142 ± 4.0 | 844 ± 2.0                       | 34.3 ± 1.3  | 57.0 ± 1.9  | 16.8 ± 0.19  | 98 ± 2.6  | 5.82 ± 0.10 |
| 3     | 6      | 931 ± 3.0        | 98 ± 1.5  | 135 ± 2.5 | 835 ± 4.2                       | 31.3 ± 0.90 | 51.4 ± 1.5  | 16.5 ± 0.20  | 94 ± 1.1  | 5.70 ± 0.08 |
| 4     | 6      | 928 ± 3.0        | 89 ± 2.0  | 128 ± 2.0 | 850 ± 5.0                       | 31.7 ± 1.3  | 50.6 ± 1.3  | 16.2 ± 0.15  | 94 ± 1.9  | 5.80 ± 0.10 |
| 5     | 6      | 929 ± 4.0        | 82 ± 1.0  | 130 ± 3.0 | 845 ± 5.0                       | 28.5 ± 0.80 | 50.6 ± 1.2  | 15.9 ± 0.23  | 93 ± 1.9  | 5.84 ± 0.07 |
| 6     | 4      | 912 ± 1.0        | 67 ± 1.2  | 125 ± 0.5 | 842 ± 4.8                       | 24.2 ± 1.5  | 50.8 ± 1.8  | 16.1 ± 0.25  | 89 ± 3.0  | 5.52 ± 0.10 |

Concentration of water, chloride and sodium in serum, and of water, chloride, sodium, non-lipoid nitrogen and potassium in fat-free brain.

tation of the data, the animals were arbitrarily divided into 6 groups depending on the concentration of chloride and sodium in the serum.

The data have been summarized in table 1. The number of animals, and the averages and respective standard errors of the individual determinations in the different groups are given.

The changes in the serum brought about by depletion of extracellular electrolyte have been described and discussed in a previous publication (1). As the table shows, there occurs a decrease in the concentrations of water, chloride and sodium. The decrease in chloride is proportionately greater than the decrease in sodium. In this study this is probably due to two factors; first, the withdrawal of a proportionately greater amount of chloride than sodium; and second, the injection of sodium sulfate solution in most of the animals in groups 5 and 6, which tended to increase the concentration of sodium slightly.

In the brain, loss of extracellular electrolyte had no effect on the quantity of total water. There was a decrease in the content of chloride and

sodium. There was a slight but statistically significant decrease in the non-lipoid nitrogen.<sup>2</sup> There was a significant decrease in the content of potassium. The decrease in brain potassium was roughly proportional to the decrease in the concentration of sodium in the serum. It will also be noted that the decrease in potassium is proportionately greater than the decrease in nitrogen. This is best shown by the changes in the ratio of potassium to nitrogen which decreases significantly as the extracellular electrolyte depletion becomes more marked.

In order to evaluate the changes in tissue electrolyte resulting from the depletion of extracellular electrolyte, we have applied the calculations previously described by Harrison, Darrow and Yannet (7) to the data just presented. These calculations are based on the assumption that all

TABLE 2

| GROUP | EXTRACELLULAR H <sub>2</sub> O<br>(PER L.) |              | BRAIN H <sub>2</sub> O (PER KG.<br>FAT-FREE TISSUE) |                    | INTRACELLULAR H <sub>2</sub> O<br>(PER L.) |              | "EXCESS" Na<br>(PER KG.<br>FAT-FREE<br>TISSUE) |
|-------|--|--------------|---|--------------------|--|--------------|--|
|       | Cl   | Na           | Extra-<br>cellular                                  | Intra-<br>cellular | Non-lipoid<br>N                            | K            |  |
|       | <i>m.eq.</i>                               | <i>m.eq.</i> | <i>cc.</i>  | <i>cc.</i>         | <i>grams</i>                               | <i>m.eq.</i> | <i>m.eq.</i>                                   |
| 1     | 131 ± 1.0                                  | 152 ± 1.2    | 293 ± 7   | 549 ± 4            | 31.0 ± 0.3                                 | 186 ± 4      | 13.1 ± 1.2                                     |
| 2     | 121 ± 1.5                                  | 146 ± 3.0    | 284 ± 10  | 560 ± 7            | 30.0 ± 0.6                                 | 173 ± 5      | 15.5 ± 1.8                                     |
| 3     | 110 ± 1.5                                  | 138 ± 2.1    | 284 ± 10  | 551 ± 5            | 30.0 ± 0.4                                 | 168 ± 2      | 12.2 ± 0.8                                     |
| 4     | 101 ± 0.5                                  | 131 ± 1.8    | 314 ± 12  | 536 ± 11           | 30.2 ± 0.7                                 | 173 ± 5      | 9.5 ± 1.2                                      |
| 5     | 93 ± 0.9                                   | 133 ± 3.2    | 306 ± 6   | 539 ± 8            | 29.5 ± 0.8                                 | 170 ± 6      | 9.9 ± 0.6                                      |
| 6     | 77 ± 0.9                                   | 130 ± 0.4    | 315 ± 12  | 527 ± 12           | 30.5 ± 0.9                                 | 167 ± 7      | 9.8 ± 1.8                                      |

Concentration of chloride and sodium in extracellular water; distribution of water; concentration of non-lipoid nitrogen and potassium in intracellular water, and quantity of "excess" sodium.

the brain chloride is extracellular and that the concentration of chloride in extracellular water may be estimated from its concentration in serum.

<sup>2</sup> Non-lipoid nitrogen represents the difference between total nitrogen and the lipid nitrogen estimated from the fat content of the brain. It had previously been determined by direct analyses of the nitrogen content of the petroleum-ether extract of the brains of approximately 50 normal cats that the average value for lipid nitrogen so determined was 2.2 grams per 100 grams of fat, with a probable error of less than 1 per cent. By using this factor for estimating lipid nitrogen rather than by direct analysis of the petroleum ether extract of the brains of the depleted animals, the introduction of a significant error pointed out by Folch and Van Slyke (6) was avoided. These authors found that from 37 to 84 per cent of the nitrogen in the petroleum ether extracts of plasma represented urea nitrogen. Since the depleted animals exhibited elevation of the non-protein nitrogen in the serum the values for lipid nitrogen of the brain, as determined by analysis of the petroleum ether extract, might have been increasingly in error as the degree of depletion became more marked. A correction was also made for non-protein nitrogen in the tissue water.

In this way it is possible to obtain an approximation of the distribution of water between the extracellular spaces and the cells, as well as the concentration of nitrogen and potassium in the intracellular water.

The data from each animal was analyzed in this manner, and the average value with its standard error determined for each of the groups of animals. The results are given in table 2. The final column gives the quantity of "excess sodium" as milliequivalents per kilogram of fat-free brain. This value represents the difference between the total sodium and the sodium present in the extracellular water, and probably represents intracellular sodium.

It will be noted that there were no significant changes in the distribution of water. The lack of any change in the quantity of intracellular water agrees with the finding of rather constant values for the concentration of

TABLE 3

*Relationships between the concentrations of sodium and chloride in the extracellular water and the content of sodium, chloride and potassium in the brain*

| VARIABLES        | CORRELATION<br>COEFFICIENT | REGRESSION EQUATION                    |
|------------------|----------------------------|--|
| $(Cl)_t, [Cl]_e$ | $0.915 \pm 0.03^*$         | $(Cl)_t = 0.24 [Cl]_e + 5.9 \pm 2.2^*$ |
| $(Na)_t, [Na]_e$ | $0.778 \pm 0.07$           | $(Na)_t = 0.36 [Na]_e + 2.8 \pm 3.1$   |
| $(K)_t, [Na]_e$  | $0.784 \pm 0.06$           | $(K)_t = 0.49 [Na]_e + 27.5 \pm 4.1$   |

$[Cl]_e, [Na]_e$  = concentration of chloride and sodium in extracellular water, respectively (m.eq. per l.).

$(Cl)_t, (Na)_t, (K)_t$  = content of chloride, sodium and potassium in the brain, respectively (m. eq. per kgm. of fat-free tissue).

\* Standard error.

intracellular nitrogen which presumably is a measure of the cellular proteins. A significant decrease can be demonstrated in the concentration of intracellular potassium. The magnitude of this change is roughly proportional to the decrease in the concentration of sodium in the extracellular water. A decrease in "excess sodium" is also observed.

By means of spot diagrams, using the data from all the animals studied, rectilinear relationships could be demonstrated between the chloride in the tissue and the concentration of chloride in the extracellular water, between the sodium in the tissue and the concentration of sodium in the extracellular water, and between the potassium in the tissue and the concentration of sodium in the extracellular water. The correlation coefficients and the regression equations expressing these relationships are given in table 3. The intercept in the regression equation expressing the relationship between the concentrations of sodium in the brain and extracellular water equals about 5 per cent of the sodium and is probably of

little significance. In the case of chloride, the intercept is more than twice the standard error and must therefore be considered significant. In the case of the normal animals, this fraction of the brain chloride represents approximately 15 per cent of the total chloride. The intercept in the equation expressing the relationship between the potassium in the brain and the concentration of sodium in extracellular water is significant, and represents approximately 25 per cent of the total potassium under normal conditions.

It should be pointed out that the regression equations given in table 3 may be considered as expressions of the respective relationships only between the ranges of concentrations of sodium and chloride in the sera of the animals studied. These varied from 47 to 120 milliequivalents of chloride and from 117 to 160 milliequivalents of sodium per liter of serum. Whether the regression lines would fit data obtained under different circumstances, or following more extensive depletion of extracellular electrolyte than can be carried out by the procedure described above cannot be predicted.

**DISCUSSION.** It is apparent that the brain cells adjust to the decrease in osmotic pressure of the extracellular fluids primarily by the release of the intracellular potassium. The nature of this adjustment is indicated by the regression equation describing the relationship between the concentrations of sodium in extracellular water and the content of potassium in the brain. It will be noted that a decrease in sodium concentration of one milliequivalent per liter of extracellular water would be associated with a decrease in potassium content of approximately 0.5 milliequivalent per kilogram of brain. Since potassium in tissues is almost entirely intracellular, and there is approximately 0.5 liter of intracellular water per kilogram of brain tissue, this would represent a decrease in the concentration of potassium in intracellular water of one milliequivalent per liter. In other words, a change in concentration of extracellular sodium is associated with an equivalent change in concentration of intracellular potassium. The intercept in the equation must therefore represent a fraction of the total potassium of the brain that plays little or no part in this mechanism of adjustment. As pointed out previously, this fraction amounts to approximately 25 per cent of the brain potassium. It is interesting in this connection to note that the concentration of sodium plus potassium in the brain water under normal conditions is 191 milliequivalents per liter as compared to a concentration of 164 milliequivalents per liter of serum water. Since the concentrations of univalent base may be considered a measure of the osmotic pressure of tissue water, it is apparent that for osmotic equilibrium to exist under these conditions, a considerable portion of the potassium in the brain must be bound in such a way as to minimize its osmotic effect. That the portion of potassium

indicated by the intercept in the regression equations represents potassium so bound is not unlikely.

The regression equations described in table 3 indicate that for all practical purposes, sodium and chloride in the brain are completely diffusible. The presence of a significant intercept in the chloride equation probably is due to the fact that the concentrations of chloride in cerebrospinal fluid are higher than estimated for extracellular water by approximately 20 milliequivalents per liter. This has been determined by direct analysis in 4 normal and 6 depleted animals. The inclusion of cerebrospinal fluid in the tissue analyzed would therefore add a constant increment to the content of tissue chloride not directly influenced by the concentration of extracellular chloride. This would necessarily appear in the intercept of the regression equation. Whether the entire intercept can be so explained must depend on the quantity of cerebrospinal fluid included in the analysis. This is a factor which could not be determined in our study. There is little evidence to believe, however, that any considerable proportion of the brain chloride is non-diffusible.

The contradictory findings described by Amberson, Nash, Mulder and Binns (8) which seemed to indicate that 80 per cent of the brain chloride was non-diffusible, are presumably explained by their failure to allow adequate time for equilibration to occur between brain and plasma before removing tissue for analysis. In the present study, the absence of a significant intercept in the equation describing the relation of sodium in tissue and extracellular fluid would indicate that an adequate interval of time had been allowed by us for this purpose.

The proportion of total chloride contributed by the red blood cells in the brain was found to be appreciably less than one per cent by analysis in 6 animals, and was, therefore, not considered in evaluating the data.

#### SUMMARY AND CONCLUSIONS

The results of the chemical analyses of the serum and brains of animals whose extracellular electrolyte were depleted to varying levels are reported. The data indicate that the brain cells adjust to this depletion by releasing intracellular potassium in amounts proportional to the decrease in the concentration of extracellular sodium. No evidence of change in total brain water or of redistribution of brain water was found.

The data presented are consistent with the view that all the sodium and probably all the chloride in the brain is diffusible.

#### REFERENCES

- (1) DARROW, D. C. AND H. YANNET. *J. Clin. Investigation* **14**: 266, 1935.
- (2) DARROW, D. C. AND H. YANNET. *J. Clin. Investigation* **15**: 419, 1936.

- (3) YANNET, H. AND D. C. DARROW. *J. Biol. Chem.* **123**: 205, 1938.
- (4) YANNET, H. AND D. C. DARROW. *J. Clin. Investigation* **17**: 87, 1938.
- (5) YANNET, H. *Arch. Neurol. and Psychiat.* **42**: 237, 1939.
- (6) FOLCH, H. AND D. D. VAN SLYKE. *J. Biol. Chem.* **129**: 539, 1939.
- (7) HARRISON, H. E., D. C. DARROW AND H. YANNET. *J. Biol. Chem.* **113**: 515, 1936.
- (8) AMBERSON, W. R., T. P. NASH, A. H. MULDER AND D. BINNS. *This Journal* **122**: 224, 1938.

## A DEMONSTRATION OF THE INDEPENDENT CONTRACTION OF THE SPHINCTER OF THE COMMON BILE DUCT IN HUMAN SUBJECTS

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Although the embryologic studies of Schwegler and Boyden (11) have demonstrated beyond question the independent origin of a distinct sphincter at the terminal portion of the common bile duct in man, a few investigators still doubt that the sphincter of Oddi functions independently of the duodenal musculature. Yet, while studying biliary pressures in choledochotomized patients, we have observed certain reactions that seem to point unmistakably to the existence of such independent activity.

**METHOD OF EXPERIMENTATION.** Five patients who had previously undergone cholecystectomy, choledochotomy and intubation of the common bile duct served as subjects for this study. An infusion flask was connected by a rubber tube to the choledochostomy tube. A recording manometer was included in the system in order to register the pressure. A flow-recording device was inserted just below the infusion flask. This device consisted of a glass bulb in which two wires had been fused in such a way as to leave a small gap, so that contact was obtained only when there was a flow of fluid. These wires were connected with a very sensitive signal magnet operated by "B" batteries. This device recorded the flow from the infusion flask through the bile ducts in such a manner that when there was a steady flow of fluid the lever of the signal magnet was held down as long as the flow continued. When the flow was intermittent, the lever recorded each interruption, and individual drops were recorded as separate excursions. This apparatus made it possible to demonstrate, on kymographic records, the production of increased resistance to the flow of fluid into the intestine as a result of sudden distention of the bile ducts. Distention was accomplished by the injection of sterile, warm, physiologic salt solution under pressure, which was attained by elevating the infusion flask. The added pressure was sustained for periods of about 60 seconds. The intraduodenal pressure was measured simultaneously by connecting a tambour to a duodenal tube attached to a balloon. The balloon was inflated with sufficient air to register duodenal contractions, but under

pressures small enough not to affect flow through the bile duct. Its position in the descending portion of the duodenum was ascertained by roentgenoscopic examination. The balloon was 12 cm. long, and it was placed so that its middle portion was at the estimated level of the papilla of Vater. The location of the papilla was known from previous cholangiographic studies. The balloon, therefore, was exposed to pressure from contractions of the duodenum for approximately 6 cm. on each side of the papilla. The duodenal tube was anchored to the face by adhesive tape. All studies were carried out after the patients had fasted for at least 8 hours.

**OBSERVATIONS.** In all of the 5 patients, when the bile ducts were suddenly distended by elevating the infusion flask to levels 40 to 50 cm. above the ducts, there were intermittent periods during the time the distention was maintained when there developed an increased resistance to the flow of fluid into the duodenum. Pain occurred throughout the period of the distention, but the pain was most intense during the periods of increased resistance to flow (1, 5). During these periods, in some but not all of the cases, there was a slight simultaneous elevation of the intraductal pressure as recorded by the manometer (*B*, fig. 1). In figure 1 these elevations are definite, amounting to an increase of 2 mm. Hg pressure within the duct. In another case, they reached a height of 6 to 10 mm. Hg; not enough, however, to indicate that the intense pain was due to distention of the duct rather than to contraction of the sphincter. The increased resistance was usually sufficient to interrupt the steady flow of fluid into the duodenum, and this was registered by the flow-recording device. In some instances, such as that represented in figure 1, the periods of increased resistance developed at fairly regular intervals, suggesting a contraction rhythm. In other instances, no such rhythm could be detected. Distention of the ducts did not alter duodenal motility. This fact was observed in every case, and may be noted in figure 1, in which contractions of the duodenum during the period of elevated pressure are shown not to differ from those before and after the interval of distention. The fact that distention of the ducts does not alter duodenal motility has been observed also in kymographic tracings made in the course of studies of the production of pain induced by sudden distention of the bile ducts (see fig. 4, reference 5). Furthermore, at the moments of decreased flow and intense pain there were no correlated changes in the intraduodenal pressure as recorded by the tambour (*C*, fig. 1). Neither was pain associated with the periods of maximum contraction of the duodenum as would be expected if the pain were caused by duodenal spasm.

**DISCUSSION.** Since the balloon was exposed to contraction of the duodenum, both above and below the ampulla, contractions registered by the apparatus are not necessarily exactly at the level of the sphincter of Oddi. Perfect correlation should not be expected in view of the mechanical

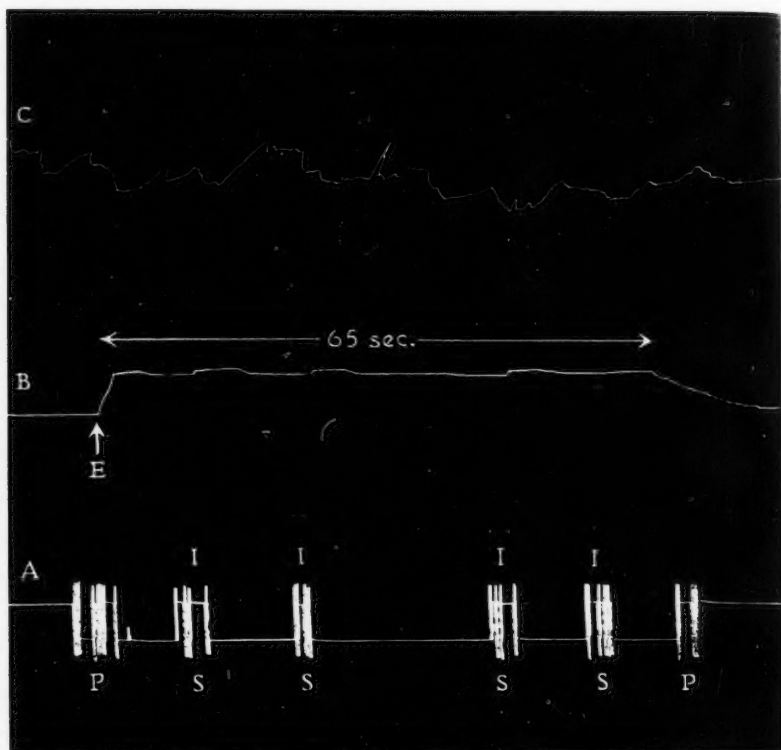


Fig. 1. A typical kymographic record of the flow of fluid through the bile duct into the intestine as recorded by a drop-counter, *A*; the pressure within the system used to distend the bile ducts, *B*; and the intraduodenal pressure, *C*. At point *E* the pressure within the distending system was raised from 15 cm. to 45 cm. of water by elevating the flask, where it was maintained for 65 seconds. When no fluid was passing through the drop-counter, the recording position was at the upper level on the graph. When individual drops flowed, separate excursions of the lever occurred. When there was a constant stream, the lever was held at the lower position, the level of simple distention. The subject experienced pain throughout the period of distention, more intense (*I*) when there was an increased resistance to flow, as revealed by a slowing of the stream to a point where individual drops could be recorded, or even to a temporary complete cessation of flow (indicated by points *S*). There was no relationship between intraduodenal pressure (*C*) and the development of the increased resistance to flow. The differences in rate of flow at the points *P* have no significance since they are due to the change in pressure caused by elevating and lowering the infusion flask.

difficulties. However, since the writing points were placed one exactly above the other, any influence upon the flow through the bile duct exerted by contraction of that part of the duodenum surrounding the balloon

should be revealed by the tracing. It is significant, therefore, that the 4 periods of spasm recorded in figure 1 do not coincide with contractions of the duodenum in the region of the ampulla or in adjacent segments of the pars descendens. Similar results were obtained in the other patients studied.

Apparently, therefore, the contraction of the duodenal musculature does not play a significant rôle in obstructing the passage of fluid into the duodenum under the conditions of this experiment. Since there is little smooth muscle in the wall of the common bile duct, except at its terminal portion, the assumption that the increased resistance to flow is due to contraction of the sphincter of Oddi appears to be justified. Furthermore, the studies of Schwegler and Boyden (11) suggest that the anatomical arrangement of the "window" through which the common bile duct enters the duodenum is such that contraction of the duodenal musculature would be much less effective in compressing the duct in man than in certain other animals (see fig. 1, reference 2). In the dog, for instance, Lueth (6) and Sandblom, Voegtlin and Ivy (10) have demonstrated that the intramural resistance to the flow of bile is definitely influenced by duodenal contraction, a physiologic observation which supports the anatomic description. In several of their experiments, however, in which the intrinsic mechanism was not masked by intestinal contraction, they demonstrated sphincteric action that was independent of duodenal tone and motility.

Finally, McGowan, Knepper, Walters and Snell (7) have demonstrated that in their group of patients with intractable post-cholecystectomy colic, spasm of the duodenum was associated with attacks of pain resembling biliary colic, and that this pain may be relieved by intubation of the common bile duct. From this, one infers that the intraductal pressure may have been elevated, although this was not measured directly. But the possibility remains that in their experiment both the pain and simultaneous rise in intraductal pressure may have been due to contraction of the sphincter within a spastic segment of the duodenum. The evidence at hand, therefore, suggests that while persistent high duodenal pressures in man may interfere with the flow of bile into the duodenum, the sphincter of Oddi can contract independently and apparently constitutes the primary occluding mechanism.

#### CONCLUSIONS

1. Distention of the common bile duct under the conditions of this experiment did not alter the existing rhythmic activity of the duodenum.
2. The intense pain induced by suddenly distending the common bile duct could be correlated only with spasm of the sphincter of Oddi.
3. In human subjects, the sphincter of Oddi can act independently of the duodenal musculature.

## REFERENCES

- (1) BERGH, G. S. AND J. A. LAYNE. *Proc. Soc. Exper. Biol. and Med.* **39**: 44, 1938.
- (2) BOYDEN, E. A. *Surgery* **1**: 25, 1937.
- (3) HENDRICKSON, W. F. *Bull. Johns Hopkins Hosp.* **9**: 221, 1898.
- (4) IVY, A. C. *Physiol. Rev.* **14**: 1, 1934.
- (5) LAYNE, J. A. AND G. S. BERGH. *Surg., Gynec. and Obst.* **70**: 18, 1940.
- (6) LUETH, H. C. *This Journal* **99**: 237, 1931.
- (7) MCGOWAN, J. M., P. A. KNEPPER, W. WALTERS AND A. M. SNELL. *Surg., Gynec. and Obst.* **66**: 979, 1938.
- (8) NUBOER, J. F. *Ztschr. f. Path.* **41**: 198, 1931.
- (9) ODDI, R. *Arch. ital. de biol.* **8**: 317, 1887.
- (10) SANDBLOM, P., W. L. VOEGTLIN AND A. C. IVY. *This Journal* **113**: 175, 1935.
- (11) SCHWEGLER, R. J., JR. AND E. A. BOYDEN. *Anat. Rec.* **67**: 441, 1937; **68**: 17, 1937; **68**: 193, 1937.

## THE RESPONSES OF THE NICTITATING MEMBRANE OF THE CAT TO CERTAIN STIMULANTS AFTER ERGOTOXINE

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Some of the effects of ergotoxine on the nictitating membrane (n.m.) of the cat have been described by Dale (1906) and by Rosenblueth (1932). This drug produces a slowly developing contraction of the n.m. which lasts unabated for at least two hours. Even small doses abolish the action potentials of the n.m. resulting from stimulation of the cervical sympathetic nerve (Rosenblueth, Leese and Lambert, 1933). The response to adrenaline, which is ordinarily a contraction, becomes a relaxation (Rosenblueth, 1932).

The present report deals with a study of the mechanical responses of the ergotoxinized n.m. to stimulation of the cervical sympathetic nerve, and to intravenous and intra-arterial administration of adrenaline, noradrenaline and acetylcholine. It brings out certain facts not predictable from data hitherto known about the n.m. and not easily explained by the current theory of the mode of action of ergotoxine.

**METHOD.** In cats anesthetized by dial (Ciba, 0.7 to 0.8 cc. per kgm., intraperitoneally) electrodes were placed peripheral to a crushed area on the cervical sympathetic chain in the neck, or, in a few experiments, on the postganglionic nerve fibers of the superior cervical ganglion. The results of the experiments were not observed to vary significantly whether pre- or postganglionic fibers were stimulated. The nerves were stimulated with tetanic frequency by means of a Harvard inductorium. Drugs were ordinarily injected into the femoral vein, but in a few experiments adrenalin (Parke, Davis) or acetylcholine (Merck) was injected into the carotid artery of the same side as the n.m. whose responses were being observed. The n.m. was attached by a thread to an isotonic lever magnifying its movements 15 to 20 times and recording on a smoked drum. After injection of the ergotoxine (Burroughs, Wellcome), strong artificial hyperventilation was necessary (cf. Dale, 1906) to prevent vascular collapse of the animal and to maintain the contraction of the n.m. due to ergotoxine. Control experiments showed that none of the effects attributed to ergotoxine were produced by hyperventilation. For the noradrenaline used in these experiments I am indebted to Dr. C. M. Greer of Vanderbilt University.

**RESULTS.** In preliminary experiments it was established that the responses of the n.m. to successive small doses of ergotoxine are cumulative and approach gradually a maximal level. This level can be easily estimated by inspection of the concentration-action curve constructed by plotting the height of response against the corresponding dose. Over 95 per cent of this maximal level was attained in 6 experiments by doses of from 0.5 to 2.5 mgm. per kgm. In the experiments reported below, the dose of ergotoxine was in all cases greater than 0.5, and usually about 3 mgm. per kgm. Thus, as judged by the contraction of the n.m., ergotoxine was producing almost 100 per cent of its effect.

*A. Responses to nervous stimulation.* Strong stimulation was necessary to elicit any response after ergotoxine. Observable responses could sometimes be obtained with frequencies of stimulation as low as 5 per second. With frequencies of 30 to 90 per second, the typical response consisted of a small, slow contraction lasting about 15 seconds and a succeeding relaxation of about the same degree below the basal level lasting 60 to 90 seconds (fig. 1C). In some experiments this relaxation was followed by a very small, delayed contraction lasting 1 to 3 minutes. In different experiments the ratio of the degree of initial contraction to that of relaxation varied widely; in some experiments there was little or no relaxation, in others, relatively little contraction. The initial contraction persisted after the largest doses of ergotoxine (4.8 mgm. per kgm.) given. The relaxing phase of the response occurred whether or not the stimulation was stopped at the end of the initial contraction, but it was more intense if the stimulation was continued. After a response to nervous stimulation, a second stimulation produced a smaller response if it occurred within 2 to 5 minutes of the first.

*B. Responses to adrenaline and to noradrenaline.* Comparatively strong doses of adrenaline were necessary to obtain responses (0.02 to 0.5 mgm. intravenously; 0.02 to 0.05 mgm. into the carotid artery). Increasing the dose of adrenaline increased the response. Typically, the response consisted of a pure relaxation lasting 1 to 3 minutes (fig. 1A). In a few experiments a very small, brief contraction occurred just before the relaxation, and in other experiments there was a very small contraction following the relaxation and lasting 1 to 3 minutes. In 3 experiments the responses of the n.m. to noradrenaline (0.5 mgm. intravenously) were identical with those produced in the same animal by adrenaline.

*C. Responses after cocaine.* In 4 animals showing the responses described in sections A and B, cocaine (6 to 8 mgm. per kgm.) was injected intravenously. The effects on the initial contraction and subsequent relaxation elicited by nervous stimulation were inconstant. In the response to adrenaline, however, an initial contraction appeared, and the relaxing phase was usually more pronounced than before cocaine. Co-

caine increased and prolonged the delayed contractions produced by either adrenaline or nervous stimulation (fig. 2). After cocaine, a further dose of ergotoxine tended to restore to the responses their pre-cocaine characteristics.

*D. Responses to acetylcholine.* When acetylcholine was injected intravenously, the response of the n.m. (fig. 1B) was similar to that resulting from stimulation of the nerves (section A). In order to rule out as much as possible the effects of sympathin and adrenaline released from distant parts of the body by acetylcholine, the latter drug was injected into the ipsilateral carotid artery in doses of 0.05 and 0.025 mgm. in 2 experiments. Here again the responses of the n.m. were similar to those resulting from

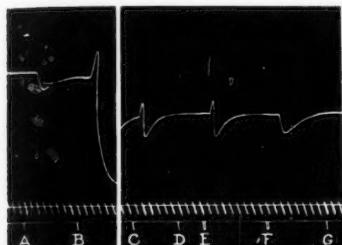


Fig. 1

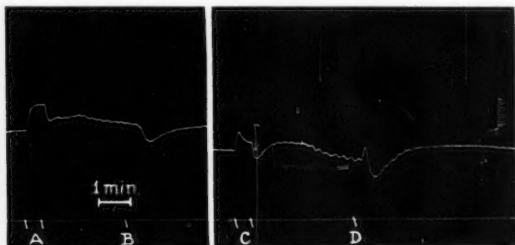


Fig. 2

Fig. 1. Typical responses of nictitating membrane of cat under dial anesthesia after ergotoxine (2.8 mgm. per kgm.).

A and F, 0.1 mgm. adrenaline, intravenously. B, 0.5 mgm. acetylcholine, intravenously. Between B and C, a lapse of 17 min. C and E, faradic stimulation with tetanic frequency of postganglionic fibers above the crushed superior cervical ganglion. D, 2 mgm. atropine. G, 1 mgm. acetylcholine. Time in 30-sec. intervals.

Fig. 2. Cat under dial anesthesia. Effects of cocaine on responses of nictitating membrane after ergotoxine (1.0 mgm. per kgm.). A and C, stimulation of postganglionic fibers by condenser discharges at a frequency of 10 per sec. B and D, 0.1 mgm. adrenaline, intravenously. Between B and C, a lapse of 10 min., during which cocaine (8 mgm. per kgm.) was given intravenously.

stimulation of the nerves. In 2 experiments, atropine (2 mgm.) abolished completely the responses to acetylcholine while leaving unaffected the responses to adrenaline and to stimulation of the nerves (fig. 1).

*E. Responses unaffected by removal of the eyeballs.* In 2 experiments the responses of the n.m. to stimulation of the nerves and to adrenaline were compared before and after careful excision of the eyeball. This operation did not change either the magnitude or the character of the responses.

*F. Responses unaffected by blood-pressure changes.* In 3 experiments the blood pressure was recorded by means of a mercury manometer. Reduction of the blood pressure by compression of the thorax showed that

the n.m. did not relax until the blood pressure fell below 40 mm. of mercury. The relaxations which resulted from this manoeuvre were rapid. In these experiments the normal vasomotor response to adrenaline was found to be reduced but not reversed after ergotoxine although the n.m. gave pure relaxations.

DISCUSSION. I. *The reversal of the response to adrenaline.* Dale's (1906) classical theory of the mode of action of ergotoxine in producing a reversal of the contracting responses of adreno-responsive organs is based on the assumption that these organs in natural conditions contain two kinds of muscular units, those which contract and those which relax in response to sympathetic nerve impulses or to adrenaline. According to this theory, ergotoxine tends to block the action of the contractile units, thus unmasking the hitherto hidden relaxing effects. This theory was shown to have a wide application and has been generally accepted.

There are, however, at least three adreno-responsive organs which show the ergotoxine reversal, yet in which evidence indicates that there is only one kind of muscular unit. 1. In the n.m. of the cat, the reversal of the response to adrenaline, if interpreted according to Dale's theory, would indicate the presence in the normal n.m. of a considerable group of relaxing muscular units. No other facts, however, support this inference, and all the known properties of the n.m. can be explained without it. 2. In the cat's uterus, Dale (1906) argued that the pregnancy reversal was a clear example of the "functional predominance" of one or the other of two pre-existent groups of muscular units, contracting or relaxing, and that the ergotoxine reversal had the same mechanism. That the uterus should contain two complete sets of muscle, one for use during pregnancy, the other for use in the non-pregnant period, appears to be an unnecessarily complex interpretation of the facts. 3. In isolated scales of the fish, *Fundulus*, Spaeth and Barbour (1917, confirmed by several other workers — e.g., Bogdanovitch, 1938) observed that ergotoxine reverses the response to adrenaline of the single melanophore cell.

These three examples are all the more cogent objections to Dale's theory because an alternative exists which explains the facts in these and most of the other organs which have been studied. This alternative theory may be stated as follows: ergotoxine tends to change the response of contractile, adreno-responsive smooth muscle units into a relaxation. It apparently originated with Anderson (see Elliott, 1905), and was favored by Bayliss (1923). Dale did not specifically consider it. Besides being especially suitable to the three organs mentioned, this theory accounts for the reversal in organs considered to contain both contractile and relaxing elements by a reversal of the response of the contracting units, so that the response of all units becomes pure relaxation.

There are, however, some organs to which the theory seems inappli-

cable. The chief of these are the rabbit's arterial system and the ileocolic sphincter of the cat (Dale, 1906), in which ergotoxine may reduce or abolish the response to adrenaline but does not reverse it. Until some reason is shown why these organs should be exceptional, this failure of reversal remains an objection to the theory advanced as an alternative to Dale's.

Neither theory, therefore, accounts for all the facts. This is not surprising in view of the increasingly apparent diversity of smooth muscles. At this stage of our knowledge, it is worth while to point out objections to a theory which has been too readily accepted, and to emphasize an alternative theory which has the advantage of being supported by direct observations of single units (melanophores of *Fundulus*). In dealing with these melanophores, the cat's uterus and the n.m. of the cat, I propose to adopt tentatively this alternative theory.

II. *Failure of reversal in the case of nervous stimulation.* The fact that ergotoxine, even in relatively high doses, fails to block completely the contractile response of the n.m. to nerve impulses, while reversing the response to adrenaline, is striking in an organ where these two types of stimulation have hitherto been regarded as interchangeable. To emphasize by contrast how regularly nerve impulses and adrenaline produce the same response in the n.m., I may report here the unusual responses recently observed by F. A. Simeone. In a certain cat under dial anesthesia, but without any other drug, the n.m. contracted in response to stimulation of the cervical sympathetic nerve, but gave a predominantly relaxing response to adrenaline (fig. 3). Among hundreds of other cats tested in this laboratory over a period of years, such a phenomenon was never before observed.

In the ergotoxinized n.m., a simple explanation of the opposition of nerve impulses and adrenaline would be that there is a fundamental difference between adrenaline and the mediator of sympathetic nerve impulses. What, then, if not adrenaline, is this mediator? The data rule out most of the other proposed mediators. The action potential will not do because even small doses of ergotoxine abolish it in the n.m. (Rosenblueth, Leese and Lambert, 1933). The argument that the mediator is not adrenaline implies also that it is not noradrenaline, for in the n.m. these two agents are affected in the same way by ergotoxine.

The similarity of the responses of the ergotoxinized n.m. to acetylcholine and to nerve impulses might suggest that the nerves producing the contractile reaction in these circumstances are cholinergic. Bacq and Fredericq (1934), finding that atropine reduces the response to nerve volleys, stated that in some cats there is a small group of cholinergic postganglionic nerves to the n.m. Atropine may, however, reduce the response of the n.m. to adrenaline (Rosenblueth, unpublished observations) and hence presumably also to sympathin. Moreover, atropine readily abolishes

the responses to acetylcholine but does not affect the response to nerve impulses (fig. 1). In the absence of other positive evidence, it seems unlikely that acetylcholine is the mediator responsible for the contractile response to nerve impulses in the ergotoximized n.m.

Previous data have shown that on the whole the response to nerve impulses is more resistant to the effects of ergotoxine than is that to adrenaline, and that this resistance to reversal differs considerably from one organ to another. Besides the organs in which the one response was as easily reversed as the other, Dale (1906) mentioned several organs in which a greater dose of ergotoxine was necessary to reverse or abolish the response to nerve impulses than was necessary for a similar change in the response to adrenaline. Moreover, Dale's observations on the iris of the cat and

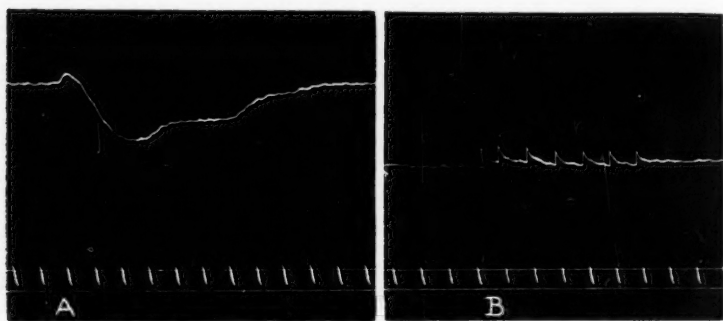


Fig. 3. Unusual responses of nictitating membrane of a cat observed by F. A. Simeone. Dial 0.75 cc. per kgm. A, adrenaline 0.015 mgm. B, stimulation of post-ganglionic nerves by groups of condenser discharges every 30 sec. Time in 30-sec. intervals.

the base of the bladder in cat and ferret, as well as Van Dyke's (1926) on the vascular bed of the cat's nasal cavity, are similar to those reported here for the n.m., in that ergotoxine failed to reverse or abolish the response to nerve volleys though reversing that to adrenaline.

If, then, one called the mediator of the n.m. sympathin and argued that it differs from adrenaline in its immunity from the effects of ergotoxine, the situation would become more complicated. For one would have to postulate a second sympathin for the vasoconstrictor system in the kidney, where reversal of the response to nerve stimulation occurs after ergotoxine, but not so readily as in the response to adrenaline, and still other sympathins for other grades of resistance to ergotoxine (cf. Barry, 1937).

A simpler explanation of these phenomena, non-committal as to subtle differences between adrenaline and sympathin, but assuming the two to be in the main quite similar, is that this difference between the effects of

nerve impulses and those of adrenaline depends on some circumstance associated with innervation which varies quantitatively from one organ to another. This is essentially the explanation proposed by Dale and Gaddum (1930) for similar facts in the action of atropine and certain parasympathetic nerves, and later adapted for 933F and the sympathetic nerves. These other cases are clearer because they do not involve either the reversal of response or the direct excitation of smooth muscle which occurs after ergotoxine.

In consideration of these factors, the following hypothesis is advanced to explain the opposition of nerve and adrenaline in the ergotoxinized n.m. While most of the muscular units would readily undergo reversal of response, as proposed in section I, certain innervated units would retain their usual contractile response; hence adrenaline, affecting all the units, would produce a relaxation, while nervous stimulation, affecting selectively the units which resist ergotoxinization, would produce an initial contraction. In other organs the difference between nerve impulses and adrenaline would be less marked because there would be a less intimate anatomical or physico-chemical relation between nerve endings and muscular units.

This theory accounts for a further feature of the observed responses to nerve impulses, namely, the relaxation which follows the initial contraction even if the faradic stimulation of the nerve continues. Here the mediator, produced by the nerve impulses in an increasing surplus, may be pictured as diffusing beyond the limits of the innervated, contracting units and, like injected adrenaline, causing relaxation in the larger number of non-innervated units. Thus if nerve stimulation continued more than a few seconds, the initial contraction would be increasingly overbalanced by relaxation—a sequence which actually occurs.

III. *The delayed contraction.* None of the theories discussed above and neither of those summarized in the last two paragraphs offer any explanation of the slight, prolonged, delayed contraction which follows the relaxing responses to both nerve impulses and adrenaline and whose augmentation is the most constant result of the injection of cocaine (fig. 2). That this response is similar after nervous stimulation and adrenaline suggests that this phase as well as the relaxing phases of the responses are of a similar nature in the two instances. Beyond this the facts do not lead us.

#### SUMMARY

1. The nictitating membrane of the ergotoxinized cat responds to adrenaline (fig. 1A) or noradrenaline with a relaxation, but to nerve impulses (fig. 1C) or acetylcholine (fig. 1B) with a contraction followed by a relaxation (p. 696).

2. Dale's theory of the mode of action of the ergotoxine reversal is

held to be inapplicable to certain organs. An alternative theory is tentatively adopted (p. 698).

3. The explanation proposed by Dale and Gaddum (1930) is adopted to account for the difference in response between nervous stimulation and adrenaline (p. 699).

#### REFERENCES

- BACQ, Z. M. AND H. FREDERICQ. *Arch. Internat. Pharmacodyn. et Therap.* 40: 294, 1934.
- BARRY, D. T. *Ibid.* 55: 385, 1937.
- BAYLISS, W. M. *The vasomotor system.* London, 1923.
- BOGDANOVITCH, S. B. *Arch. Internat. Pharmacodyn. et Therap.* 59: 227, 1938.
- DALE, H. H. *J. Physiol.* 34: 163, 1906.
- DALE, H. H. AND J. H. GADDUM. *Ibid.* 70: 109, 1930.
- ELLIOTT, T. R. *Ibid.* 32: 401, 1905.
- ROSENBLUETH, A. *This Journal* 100: 443, 1932.
- Unpublished observations.
- ROSENBLUETH, A., C. LEESE AND E. LAMBERT. *This Journal* 103: 659, 1933.
- SPAETH, R. A. AND H. G. BARBOUR. *J. Pharmacol. Exper. Therap.* 9: 431, 1917.
- VAN DYKE, H. B. *Ibid.* 27: 299, 1926.

## THE PRODUCTION OF UNCOMPLICATED RIBOFLAVIN DEFICIENCY IN THE DOG<sup>1</sup>

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It has been demonstrated that thiamin (1), nicotinic acid (2), vitamin B<sub>6</sub> (3), and factor W (4) are essential for the nutrition of the dog. In addition, other members of the vitamin B complex may be required (4). Only the thiamin and nicotinic acid requirements have been quantitatively determined (5, 6, 7, 8). Street and Cowgill (9) have expressed the riboflavin requirement of the adult dog as 25 micrograms per kilogram of body weight per day. However, since the food intake of their experimental dogs was restricted and the animals lost weight throughout the experiment, the requirement as stated by these authors cannot be considered final.

In an effort to determine the quantitative requirement of the growing dog for riboflavin, and in order to obtain a picture of uncomplicated riboflavin deficiency, we have endeavored to construct a ration devoid of this vitamin, yet complete with respect to the other factors required by the dog. We have used a synthetic basal ration similar to that of the other workers (10). This has been supplemented with synthetic thiamin, nicotinic acid, and vitamin B<sub>6</sub>. The remaining factors required by the dog have been furnished by a purified liver concentrate freed from riboflavin. This ration has been shown to give good growth for the dog when supplemented with riboflavin (4). In the absence of added riboflavin, an acute deficiency is produced in 6 to 8 weeks.

**METHODS.** The basal ration was essentially that used by Street (10) and has the following composition:

|  |          |
|--|----------|
| Purified casein (washed 8 times, reprecipitated twice).... | 19 grams |
| Sucrose.....   | 66 grams |
| Cotton seed oil.....                                       | 8 grams  |
| Cod liver oil.....   | 3 grams  |
| Salts 3 (4).....   | 4 grams  |

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<sup>2</sup> Commercial Solvents Corporation Fellow.

|                              |                |
|------------------------------|----------------|
| Thiamin.....                 | 300 micrograms |
| Nicotinic acid.....          | 5 milligrams   |
| Vitamin B <sub>6</sub> ..... | 300 micrograms |
| Haliver oil.....             | 2 cc. per week |

Acid acetone or hexane-butanol filtrate  $\approx$  4 grams of liver extract.

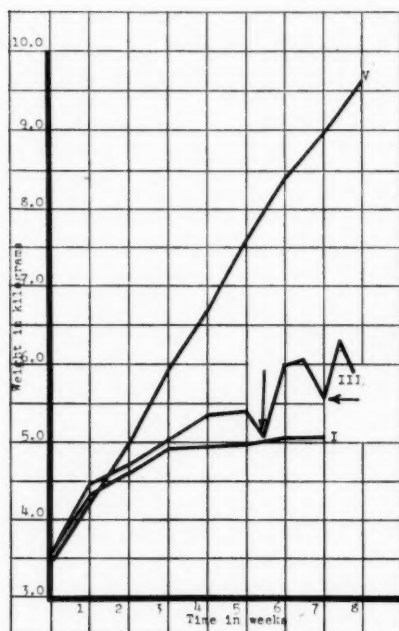


Fig. 1

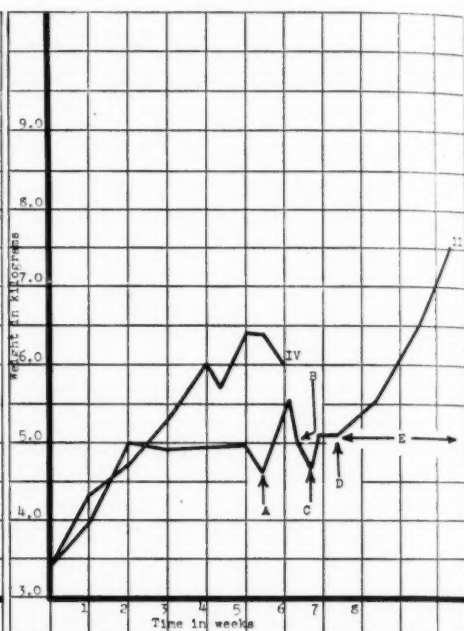


Fig. 2

Fig. 1. Growth of dogs on riboflavin deficient ration. Dog I. Basal ration. Dog V. Basal ration plus concentrate equivalent to 200 micrograms of riboflavin per 100 grams of ration. Dog III. Basal ration. Arrows indicate oral administration of riboflavin at a level of 100 micrograms per kilogram of body weight.

Fig. 2. Growth of dogs on riboflavin deficient ration. Dog II. Basal ration. A and B: oral administration of 100 micrograms per kilogram of body weight. C: 400 micrograms of riboflavin subcutaneously. D: 1 milligram of riboflavin subcutaneously. E: 400 micrograms of riboflavin per 100 grams of ration administered daily. Dog IV. Basal ration plus 200 micrograms of synthetic riboflavin per 100 grams of ration, administered by mouth in a single weekly dose.

A liver extract remaining after the removal of the P.A. factor was supplied by Dr. David Klein, Wilson Laboratories, Chicago, and was employed as the source of both filtrates. The acid-acetone extract was prepared according to the method of Frost and Elvehjem (11). The

hexane-butanol extract was prepared according to Black, Frost and Elvehjem (12). Riboflavin was removed from these concentrates by adsorbing three times on fuller's earth at pH 1 to 2. The fuller's earth filtrate was neutralized and fed at a level equivalent to 4 per cent of the original liver extract. At this level, all of the filtrate factors necessary for the dog are adequately furnished. The flavin-free concentrates contained less than 0.02 microgram of riboflavin per gram of liver extract when assayed by a microbiological technique (13).

Synthetic riboflavin was administered either orally or by subcutaneous injection. Two similar natural riboflavin concentrates containing 2000 and 2700 micrograms of riboflavin per gram<sup>3</sup> were employed in some experiments. These were administered orally.

**RESULTS.** Growth records of the dogs upon the basal ration plus various supplements are shown in figures 1 and 2. Detailed observations upon the individual dogs are given in the following summaries.

*Dog I.* This animal was given only the basal ration with the acid-acetone filtrate. A normal growth response for 2 weeks was followed by a weight plateau until death. Food consumption after the first 2 weeks dropped below that of the control animals, but was maintained at an average daily intake of 150 grams until the onset of collapse. This is in agreement with the observations of Street and Cowgill (9). The characteristic sudden collapse noted by previous workers (9, 14) was also observed. Within a period of 3 hours, the animal lost the ability to stand, became comatose, and died. Necropsy showed no significant abnormalities in the heart, kidney or liver. However, large amounts of blood were found throughout the pyloric end of the stomach and the small and large intestines. Gross examination indicated a mild inflammation of the stomach mucosa and severe ulceration throughout the duodenum, jejunum, and ileum. In all, more than 35 eroded areas were observed. The severity of the intestinal hemorrhage makes it appear likely that this was the immediate cause of death.

*Dog II.* This animal received the basal ration with the acid-acetone filtrate. After a short growth period, the usual weight plateau was observed (3 weeks). At the first sign of significant weight loss, a single oral dose of 100 micrograms of riboflavin per kilogram of body weight was administered. At this time the dog was lethargic but otherwise normal. A weight response of 1 kgm. in 5 days was noted. This was followed by a rapid weight loss of 700 grams. Another single dose of 100 micrograms of riboflavin per kilogram was administered but caused no weight response. The dog was, therefore, injected subcutaneously with 400 micrograms of

<sup>3</sup>These samples were kindly supplied by Commercial Solvents Corporation, Terre Haute, Indiana. We are indebted to Merck and Company, Rahway, New Jersey, for generous supplies of thiamin, nicotinic acid, and vitamin B<sub>6</sub>.

riboflavin and showed immediate improvement. After 1 week growth ceased and 1 mgm. of riboflavin was injected subcutaneously. Growth was immediately resumed. Four hundred micrograms of riboflavin per 100 grams of ration were then administered daily by pipette and a rapid rate of growth ensued. After 1 week, the hexane-butanol filtrate was substituted for the acid-acetone filtrate, but no change in the rate of growth was observed. The animal continued to receive 400 micrograms of riboflavin per 100 grams of ration for 1 month and showed excellent growth throughout this period.

*Dog III.* This dog was depleted on the basal ration plus the acid-acetone filtrate for 5 weeks. The usual weight plateau was noted. At the first significant weight loss, a natural riboflavin concentrate was administered in a single dose at a level equivalent to 100 micrograms of riboflavin per kilogram of body weight. A weight response of 900 grams in 5 days was noted. This is similar to the response observed with an equivalent amount of synthetic riboflavin. A rapid weight loss of 500 grams then occurred. A second similar dose of riboflavin concentrate resulted in a weight response of 600 grams. This was followed by rapid weight loss and the typical collapse syndrome. Necropsy showed symptoms identical to those observed in dog I. The intestine was turgid with congealed blood, and more than 20 eroded areas were observed throughout the duodenum, ileum and jejunum.

*Dog IV.* This animal was maintained on the basal ration plus the acid-acetone filtrate. In addition 200 micrograms of riboflavin per 100 grams of ration were fed weekly in a single dose. This was administered in the form of an aqueous suspension containing 400 micrograms of riboflavin per cubic centimeter. This amount permitted a slow rate of growth, but death ensued after 5 weeks. The usual sudden collapse characteristic of riboflavin deficiency was noted, the dog dying within 4 hours after the first signs of lethargy and weakness. Gross examination of the organs indicated no pathology in the lungs, heart, and liver. The spleen was pale and small. The mucosa of the pyloric end of the stomach and the duodenum was inflamed.

*Dog V.* This animal was maintained on the basal ration plus the acid-acetone filtrate supplemented with 200 micrograms of riboflavin per 100 grams of ration. The riboflavin was fed in the form of the natural concentrate. A normal rate of growth was obtained and the animal remained healthy throughout the experiment.

**DISCUSSION.** Early studies upon the production of experimental riboflavin deficiency in the dog appear to have been complicated by a number of factors. Sebrell and Onstott (14) produced this deficiency in adult dogs receiving a purified ration supplemented with 25 per cent of rice polishings

as a source of the B vitamins. The development of a hypochromic anemia in some of the animals which was not cured by the administration of riboflavin suggests a complicating deficiency. The ration of Street and Cowgill (9), containing an extract of rice polishings fed at a level equivalent to 1 gram of rice polishings per kilogram of body weight per day as a source of the B complex, is probably subject to the same complicating deficiencies. The fact that 1 gram of this extract per day permits good growth in rats is no indication that it will be adequate in the B vitamins for the dog when fed at approximately one-fifth of this level per unit of body weight. It should also be emphasized that the B complex requirements of the dog may differ greatly from those of the rat. This is particularly evident in the case of nicotinic acid.

We have preferred to use liver concentrates as a source of certain members of the B complex since these materials have been repeatedly tested in this laboratory and have been shown to contain adequate amounts of the filtrate factors. In addition, we have used growing dogs as experimental animals, since such animals succumb to the vitamin deficiency much more rapidly than do adult animals. Finally, growing animals permit much more rigorous testing of the experimental ration with regard to adequacy of the other factors required by the dog. Our data, coupled with those of McKibbin, Madden, Black and Elvehjem (4), demonstrate that the basal Street ration supplemented with thiamin, vitamin B<sub>6</sub>, nicotinic acid, the acid-acetone or hexane-butanol filtrates, and riboflavin is nutritionally adequate for the growing dog. Normal growth is maintained on such a dietary regimen. When riboflavin is omitted from the ration, the characteristic collapse syndrome and sudden death are observed in 6 to 8 weeks.

Our experimental data thus far do not permit an accurate definition of the riboflavin requirement of the growing dog. The results in the case of dog IV indicate that 200 micrograms of riboflavin per 100 grams of ration do not satisfy the requirement of the animal. However, it should be pointed out that the vitamin was administered in a single dose weekly and may not have been efficiently absorbed or utilized. Evidence for this view may be derived from the results with dog V. This animal received the riboflavin concentrate at a level equivalent to that of dog IV and showed excellent growth throughout the experimental period. This concentrate was added to the basal ration weekly and was consumed over a period of several hours. It is possible that the slow consumption under these conditions resulted in more complete absorption or utilization of the vitamin than in the case of dog IV. The possibility that the natural concentrate furnished essential factors in addition to riboflavin is not likely since the ration has been shown to be nutritionally adequate except for riboflavin. In addition the extremely high potency of this concentrate

with respect to riboflavin permitted feeding at a level of approximately 1 gram weekly. At this level it is unlikely that accessory factors required by the dog would be furnished by the riboflavin concentrate.

The results with dog II showed that 400 micrograms of riboflavin per 100 grams of ration permits a normal rate of growth and maintains the animal in good health.

The severe ulceration observed in two cases of riboflavin deficiency is interesting, but its significance cannot yet be determined. Further studies are in progress upon the determination of the riboflavin requirement of the growing dog using preventive assays. In addition, more complete studies of the specific pathology associated with riboflavin deficiency will be reported.

#### SUMMARY

The basal ration of Street (10) has been shown to be adequate for the growing dog when supplemented with thiamin, nicotinic acid, vitamin B<sub>6</sub>, the acid-acetone or hexane-butanol filtrate of liver extract, and riboflavin. In the absence of added riboflavin, acute deficiency is produced in 6 to 8 weeks.

#### REFERENCES

- (1) COWGILL, G. R. The vitamin B requirement of man. New Haven, Yale University Press, 1934.
- (2) ELVEHJEM, C. A., R. J. MADDEN, F. M. STRONG AND D. W. WOOLLEY. *J. Biol. Chem.* **123**: 137, 1938.
- (3) FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES. *J. Nutrition* **16**: 197, 1938.
- (4) MCKIBBIN, J. M., R. J. MADDEN, S. BLACK AND C. A. ELVEHJEM. *This Journal* (in press).
- (5) ARNOLD, A. AND C. A. ELVEHJEM. *J. Am. Vet. Med. Assn.* **95**: 187, 1939.
- (6) SEBRELL, W. H., R. H. ONSTOTT, H. F. FRASER AND F. S. DAFT. *J. Nutrition* **16**: 355, 1938.
- (7) MARGOLIS, G., L. H. MARGOLIS AND S. G. SMITH. *J. Nutrition* **16**: 541, 1938.
- (8) BIRCH, T. W. *J. Nutrition* **17**: 281, 1939.
- (9) STREET, H. R. AND G. R. COWGILL. *This Journal* **125**: 323, 1939.
- (10) STREET, H. R. *Proc. Soc. Exper. Biol. and Med.* **36**: 602, 1937.
- (11) FROST, D. V. AND C. A. ELVEHJEM. *J. Biol. Chem.* **121**: 255, 1937.
- (12) BLACK, S., D. V. FROST AND C. A. ELVEHJEM. *J. Biol. Chem.* (in press).
- (13) SNELL, E. E. AND F. M. STRONG. *Ind. and Eng. Chem. (Analyt. Ed.)*, **11**: 346, 1939.
- (14) SEBRELL, W. H. AND R. H. ONSTOTT. *U. S. Pub. Health Repts.*, **53**: 83, 1938.

## THE DYNAMICS OF THE FROG AND TURTLE HEARTS—THE NON-REFRACTORY PHASE OF SYSTOLE

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The dynamics of the heart beat and circulation in the frog and turtle has not received the study it deserves. Many questions of general biological and physiological interest are, however, contingent upon such knowledge. What systolic and diastolic pressures exist in the aorta? Is the peripheral resistance high enough to allow gradual efflux from the arterial reservoirs during diastole? Are the semilunar valves effective in preventing regurgitation during diastole? Are the pulmonary and arterial pressures, created by action of a single ventricle, controlled differentially, and if so, by what mechanism? Is the pulmonary division of the aorta shut off from the ventricle before the systemic by contraction of the muscular ring? Does the ventricle have an isometric phase? If so, is it constant, and what is its duration? Where are the beginning and end of mechanical systole,—a question of fundamental importance in correlating electrical and mechanical changes and in limiting the end of the refractory phase? If an isometric phase exists, mechanograms registering the shortening of muscle may not be accepted as denoting the start of contraction, and it is highly questionable whether the peaks of contraction curves used to study the refractory period denote the end of systole.

These and similar questions could be answered by simultaneous optical pressure curves from the ventricle and aorta; but until the development of new principles for use of manometers with small cannulae by Hamilton, such curves were not easily obtainable.

**METHODS.** Ventricular and aortic pressure curves were recorded by means of the Gregg type of optical manometer. Large bullfrogs and medium sized turtles were pithed with insignificant loss of blood. The heart was exposed in the customary manner. Ventricular pressure was recorded by inserting a short 18 to 20 gauge hypodermic needle through the ventricular wall, care being taken to fix it so that the end was free in the cavity. Aortic pressure was recorded through a small cannula tied into one systemic branch of the truncus arteriosus. The manometers

<sup>1</sup> Crile Summer Scholar for 1939.

were calibrated under static conditions, in the customary manner, with reference to a standard base line.

*Normal pressure pulses.* Typical records of intraventricular and aortic pressures from normal hearts in good condition are shown in figure 1, marked with pressure and time values. The two on the left are from turtles; those on the right from bullfrogs. They exemplify the various forms of pressure curves both from frogs and turtles. In general, records from the turtle hearts indicate a superior dynamic contraction.

A casual inspection shows an unanticipated similarity in forms to those recorded from cats and dogs, including the presence of a definite isometric period, and a small but definite aortic incisura during closure of the semilunar valves. As in experimental dogs and cats also, the configuration varies somewhat. Occasionally, atrial contraction causes a definite wave, as in the upper tracings; more generally this is barely shown, as in the lower ones. Since such curves were recorded when the atria were seen to empty visibly during contraction, it appears that the presence or absence of such waves is determined chiefly by the distensibility of the ventricle at this time.

The systolic pressures in the ventricle and aorta were essentially the same and in vigorously beating hearts ranged from 26 to 34 mm. The aortic curve shows that the pressure continues to drop gradually during the long diastole and that peripheral resistance is sufficient to maintain a diastolic pressure of about 15 mm. Hg ( $\pm 3$ ). Thus, the pulse pressure is about 15 mm. Hg.

At normal heart rates of about 30 per min., the total duration of systole varied considerably in different hearts, tending to be slightly less in frogs—which may be accidental. Isometric contraction varied from 0.06 to 0.13 sec. in most of the curves; occasionally it was longer as shown in the lower right curve of figure 1, where it equals 0.18 sec. In one experiment with a very low diastolic pressure it was scarcely measurable. The experiments indicate that enough variation occurs in the isometric phase in different animals and in the same heart over a period of time to be important in comparison of electrical and mechanical events. The proto-diastolic phase, denoting closure time of semilunar valves, is very short, generally 0.04 to 0.05 sec. The isometric relaxation is definitely longer than isometric contraction.

*Optimal heart rates.* It has been established reasonably well in normally innervated hearts of dogs under conditions assuring normal *effective* venous pressures, that progressive acceleration above basal rates increases the minute output and mean blood pressure. The optimal rate is high (ca. 180-200 per min.), i.e., above these frequencies further increase is accompanied by diminished minute output and fall of blood pressure.

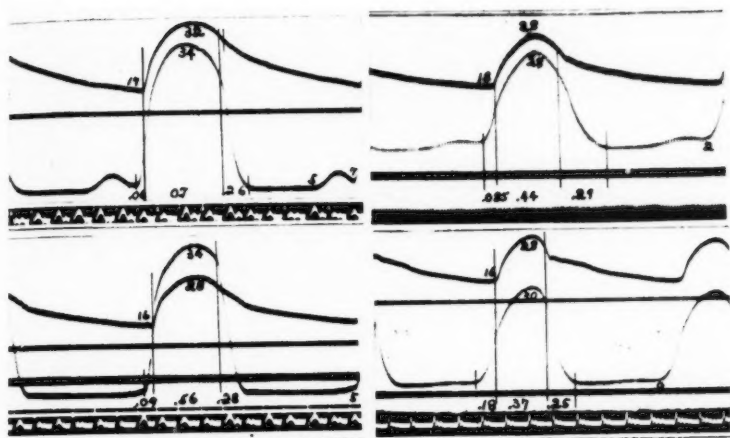


FIGURE 1

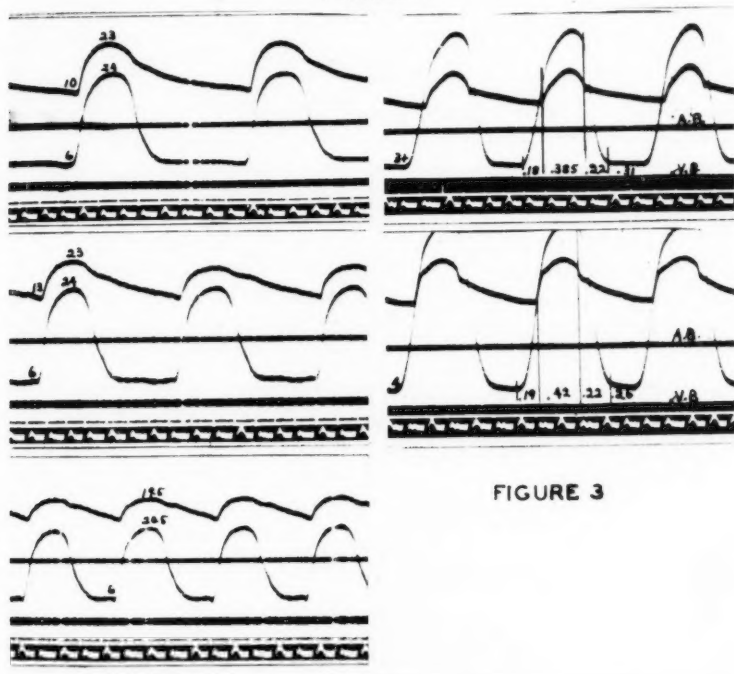


FIGURE 3

FIGURE 2

Increase in minute output up to optimal rates is accompanied by an increase in diastolic pressure and decrease in pulse pressure.

When the hearts of turtles—beating about 30 per min. in summer—are artificially accelerated by artificial break shocks applied to the right atrium, the diastolic pressure rises slightly but the systolic is not affected up to increases around 40 per min. and beyond that actually falls. Figure 2 shows segments of such records at heart rates of about 30, 40 and 72 per minute respectively. It appears that the optimum heart rate is very low, and we have seen no evidence that the intact turtle heart possesses an effective mechanism for increasing minute output by increase in heart rate.

*Infusion of Ringer's solution.* The response of the intact ventricles to increased distention is easily tested by filling the manometer system with Ringer's solution and introducing this directly into the ventricle. Many tests showed that as the ventricle visibly distends, initial pressure is likewise increased. An illustration is shown in figure 3. In the lower curve, after infusion, initial tension is slightly increased with a visibly larger size and larger systolic discharge, as judged by the pressure pulses.

As in mammalian hearts, the duration of contractions is prolonged, while the isometric contraction remains unchanged provided diastolic aortic pressure remains reasonably constant. At constant heart rates this lengthening of systole occurs at the expense of diastole and particularly the latter part of the inflow phase, as clearly shown by comparing the two curves of figure 3.

*Limitations of the refractory phase.* Since the earliest experiments of Kronecker and Marey it has become standard teaching in physiology, and has supposedly been confirmed innumerable times in teaching laboratories, that the frog and turtle ventricle is absolutely refractory to stimuli up to the very end of systole. In 1925, one of us (W) showed that this is not true of the dog's ventricles, which respond to strong induction shocks applied up to 0.04 to 0.09 sec. before the end of systole, the exact limit being determined by duration of systole and heart rate. The possibility that mechanograms generally used to test these responses may not indicate the end of systole, made it desirable to restudy the potency of induction shocks during systole, using pressure pulses as criteria. Accordingly, break induction shocks from a Harvard inductorium actuated by 4 volts were introduced every fifth beat by a rotatory exciter, in such a way that the shocks were slightly advanced in the heart cycle each time. Selected beats from a more complete series are reproduced in figure 4. It will be observed in segments *F*, *G* and *H* that shocks given during the last third of systole, denoted by the incisura, evoke a contraction starting on the descending limb of the pressure curve. Similar effects were obtained when direct currents of brief duration (ca. 0.05 to 0.12 sec.) were applied through the electrodes in alternating directions in successive shocks. The lower record of figure 4 illustrates such an effect.

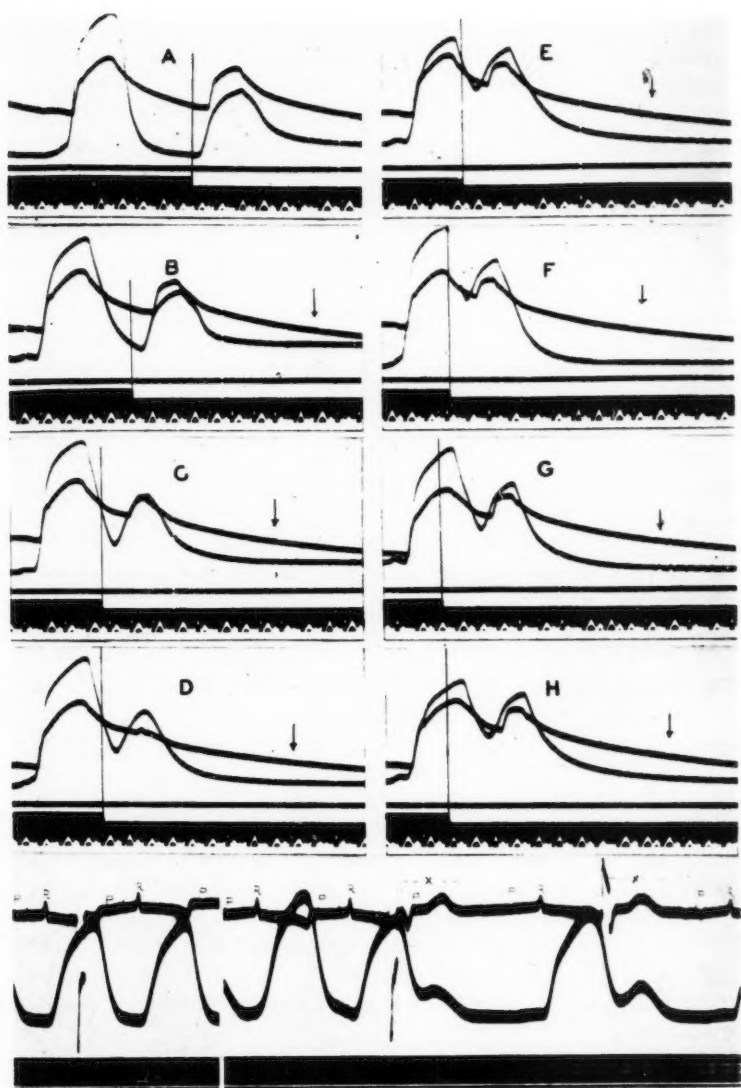


Fig. 4

The question may be raised whether the premature ventricular contractions elicited by shocks applied during systole may not have been due to spread of current to the atria and later excitation of the ventricle by an atrial impulse. The fact that the ventricular beats showed a full com-

pensatory pause and that the longest latent period is definitely less than the normal  $A_sV_s$  interval are circumstantial though not crucial proof that this did not occur in these experiments. Unquestioned evidence is supplied in the lowest record of figure 4 in which a lead III E.C.G. was simultaneously recorded. When a shock during systole or during diastole elicits the premature contraction it is accompanied by an electrographic wave (X) of undisputed ventricular origin. Furthermore, the sequence of P waves—small but distinct in the original records—is not disturbed and maintenance of normal atrial rhythm could easily be verified by direct observation.

The vigor of the premature beats shown in segments *E-H* and their accompaniment by expulsion of blood as indicated by the rise of aortic pressure is extraordinary, when we consider that they occur before any renewed filling of the ventricle could have occurred. The only conclusion that can be drawn is that the ventricular emptying during normal beats is far from complete; in fact it retains sufficient blood for another good ejection without refilling. This differs from the mammalian ventricle in which the residual blood is small.

The presence of such residual blood together with the response of the ventricle to increased initial length provide the mechanism by which minute output can be increased.

#### SUMMARY AND CONCLUSIONS

Pressure pulses from the ventricle and aorta of bullfrogs and turtles were recorded by Gregg optical manometers and the following facts regarding the dynamics of these cold-blooded hearts were established:

1. Intraventricular and aortic pressures resemble those recorded from cats and dogs in form and phasic subdivision. The presence of an isometric period ranging from 0.06 to 0.13 second, the occurrence of a definite incisura, and the differing diastolic gradients in ventricle and aorta indicate efficient valvular action at the A-V and aortic orifices.

2. Systolic aortic pressures range from 26 to 34 mm., diastolic from 12 to 18 mm., giving an average pulse pressure of about 15 mm. The slow diastolic gradient indicates that an adequate peripheral resistance allows a gradual drainage of blood from the elastic reservoirs during diastole as in mammals.

3. Like mammalian hearts, the intact ventricle responds to increase in initial tension and length by increase in output and lengthening of systole. Deviations of initial tension and length were not observed.

4. Like the mammalian ventricle also, the ventricle of the turtle (and presumably the frog which was not tested) is not refractory throughout systole; on the contrary, the latter third of systole is responsive to strong induction shocks and brief direct currents. The occurrence of ventricular

complexes in electrocardiograms and preservation of the natural tempo of the atria guarantee that these results are not artefacts.

5. The turtle's and frog's ventricle, unlike those of mammals, does not increase the minute output by increase in rate, but beats at an optimum systolic discharge at rates of 30 to 40 per sec. Further increase in rates cause progressive reduction in minute volume with decline in systolic blood pressure.

6. Unlike the mammalian ventricles also, those of the turtle and frog retain a substantial residual volume of blood at the end of each ejection,—sufficient to cause a significant ejection in a premature contraction beginning during the isometric relaxation phase.

## THE ANTI-PRESSOR ACTION OF RENAL EXTRACTS AND THEIR CAPACITY TO REDUCE THE BLOOD PRESSURE OF HYPERTENSIVE RATS<sup>1</sup>

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Recent studies indicate that many cases of hypertension as produced experimentally or observed clinically are due to renal dysfunction. The available evidence points to the possible formation by the diseased kidney of a pressor substance but also indicates that normal renal tissue may play a rôle in eliminating the hypertensive effects of the diseased organ. In the present paper two general methods have been pursued to test the hypothesis that the normal kidney produces a humoral agent which is capable of abolishing hypertension. An attempt has been made to inhibit the pressor effects of renin and other pressor substances by the administration of renal extracts and these extracts have been used to reduce the blood pressure of hypertensive animals.

Tigerstedt and Bergman (1), in their original communication describing renin (the renal pressor substance), pointed out that its pressor action was more marked and more prolonged in rabbits which had been subjected to nephrectomy two days previously than in normal rabbits. They attributed this difference to the inability of the nephrectomized animals to excrete the injected renin. In repeating their experiments, Merrill, Williams and Harrison (2) found that the increased sensitivity to renin did not appear immediately after nephrectomy but developed two or more days later. These experiments showed that a failure of the kidney to excrete the injected renin could not be responsible for the difference observed. One possible interpretation of these findings is the assumption that the body normally contains some substance elaborated by the kidneys which antagonizes the pressor action of renin, and which gradually disappears from the body after the removal of the kidneys. The purpose of the following experiments was to test this hypothesis.

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**METHODS.** A number of procedures were evolved for preparing from the kidneys of hogs a sufficiently purified and concentrated extract containing the hypothetical renal anti-pressor substance. In view of the complexity of the technical details involved in the preparation of the extract, the description of the process will be reserved for a more appropriate journal.

Rats were used as test objects in all the experiments. The animals were anesthetized with sodium pentobarbital administered intraperitoneally. Blood pressure was measured by cannulation of the abdominal aorta, the technique used being that described in a previous communication (3). Purified heparin (obtained from the Connaught Laboratories) was used as an anti-coagulant. After the blood pressure had been constant for a period of several minutes renin was administered through the aortic cannula. The renin was prepared according to the method described by Grossman (4), except that the alcohol-insoluble fraction was extracted with 0.9 per cent saline instead of sodium bicarbonate solution. The dose employed was calculated according to the formula

$$\frac{2 \times \text{body weight in grams}^{2/3}}{100} = \text{cubic centimeters administered}$$

This dosage was employed because previous experience (3) had shown it to be a convenient one.

In one group of experiments renal extract which had been freed of pressor activity was administered through the aortic cannula in doses of 0.25 to 1.0 cc. Five minutes later renin was injected by a similar route and the pressor response of a series of animals so treated was compared to that of other rats receiving renin alone, or saline solution followed by renin. In a second and larger series of experiments, two groups of rats were matched for sex and weight. One group received only the stock diet; the other group received the stock diet in which a measured amount of renal extract had been incorporated. After a period of two to four days the pressor response to renin was compared in the two groups, according to the technique mentioned above. A total of ten experiments was carried out, each including observations on three to six controls and an equal number of animals which had received the anti-pressor fraction. In two experiments the anti-pressor renal extract was injected intra-arterially, and in the remaining eight instances it was administered orally.

In a third series of experiments animals which had received renal extract orally were compared with controls as regards their pressor response to ephedrin, epinephrin, and pitressin.

**RESULTS.** It was found that certain preparations of renal extracts yielded fractions which had no significant effect on the blood pressure of

the animals but caused well marked inhibition of the pressor action of subsequently injected renin. Such inhibition was obtained not only when the extracts were administered by injection but also when the oral route was employed (fig. 1). In each instance a well marked depression of the pressor response to renin occurred. The maximum rise in blood pressure was less and the return to the normal level was considerably more rapid than in the case of the untreated controls receiving an equal amount of

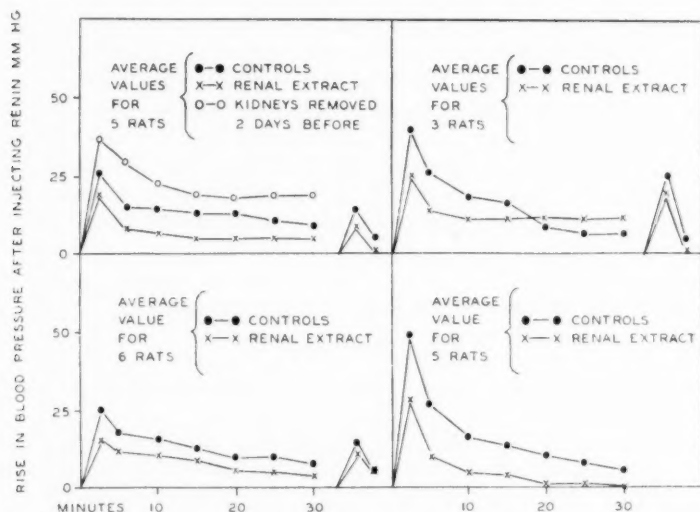


Fig. 1. Effect of oral administration of renal extract on pressure response to renin. In one experiment the pressor effect of renin in a group of rats which had been subjected to nephrectomy two days previously, was shown to be definitely greater than that of a group of normal rats. (This confirms our previous findings (2).) In four experiments rats which had received renal extract mixed with their food were found to be less sensitive to renin than were the controls which had been fed the stock diet. (In computing the average rises in blood pressure, values less than the pre-injection level were neglected.)

renin. It was not usually possible to abolish completely the pressor effect of renin, although complete inhibition was observed in one rat. However, a diminution in the response to renin was regularly observed.

In the course of the experiments a number of pregnant rats were encountered. It was noted that such animals resembled those receiving renal extract in that they were abnormally insensitive to renin (fig. 2).

In four experiments the effects of ephedrin and pitressin were studied. In each instance the pressor response to ephedrin was less in the animals which had received renal extract than in the controls. In two experiments

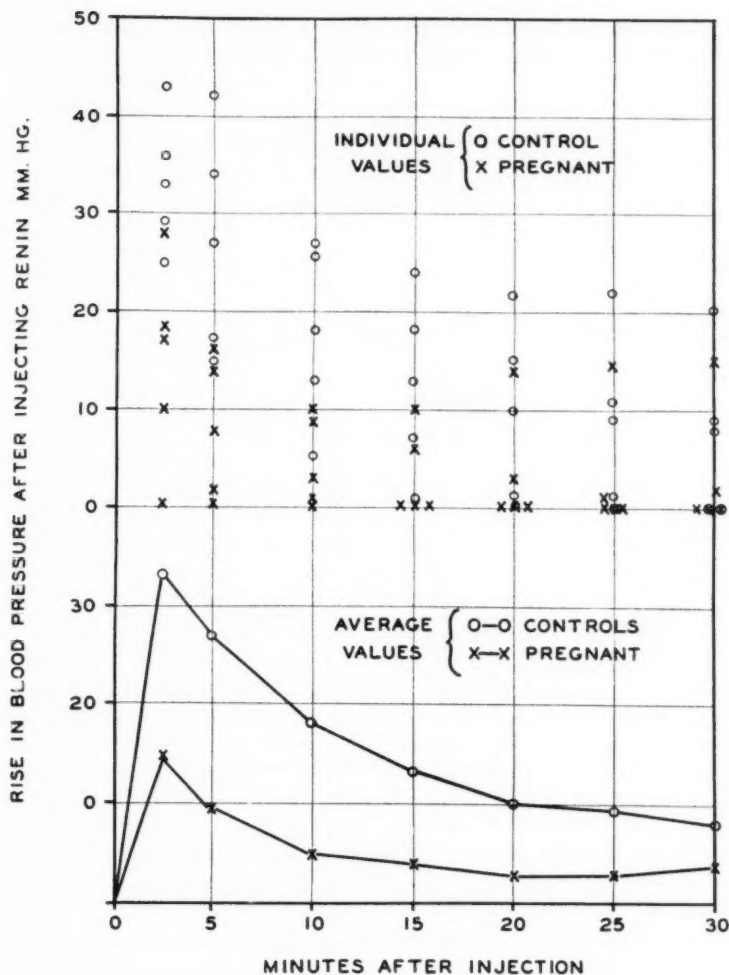


Fig. 2. Effect of pregnancy on the pressor response to renin. Five normal pregnant rats and five normal non-pregnant female rats were anesthetized with sodium pentobarbital. After cannulation of the abdominal aorta the average blood pressures were 115 and 109 mm. of mercury for the control and pregnant animals, respectively. Following the injection of renin in comparable doses the increase in blood pressure produced in the pregnant rats was distinctly less than in the controls.

the treated animals were less sensitive to pitressin while in the two other observations no difference was noted. Inconclusive results were obtained in the two experiments in which epinephrin was injected.

*The effect of renal extracts on the blood pressure of hypertensive rats.* In order to study the effects of anti-pressor extracts on the blood pressure of

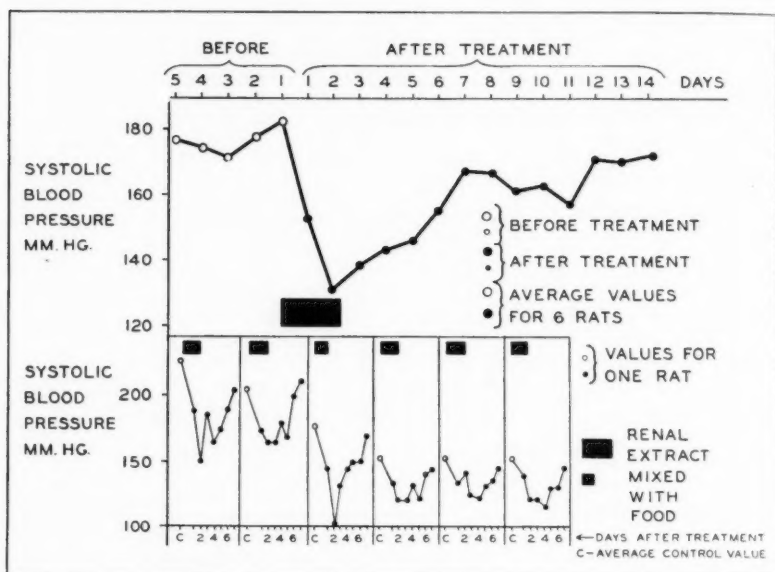


Fig. 3. The administration of renal extract orally was followed by a well marked decline in blood pressure, which gradually returned to normal after the extract was withdrawn. The upper curve represents the average value for six rats. Each of the lower curves depicts the blood pressure of a single rat.

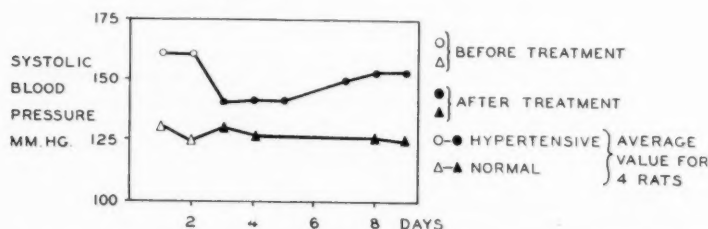


Fig. 4. Comparison of response of normal and hypertensive rats to renal extract administered orally. The administration—per os—of renal extract caused a decline in blood pressure in the hypertensive rats. No significant change occurred in the blood pressure of the normal rats. Note: The normal rats received four times as much renal extract as the hypertensive rats.

animals with hypertension, the procedure described by Chanutin and Ferris (5) was employed, blood pressure being measured without anesthesia according to the technique recently described (6). Most of the rats

developed a well marked elevation in blood pressure within a few weeks after subtotal nephrectomy. Renal extracts containing the anti-pressor agent were then administered either intraperitoneally or orally. In the earlier experiments the intraperitoneal route was employed almost exclusively. Although satisfactory reductions of blood pressure usually occurred, toxic effects due to impurities frequently appeared and it was uncertain whether such toxicity might not account for the observed decline in blood pressure. This objection was overcome by means of oral administration. With adequate doses of properly prepared extracts it has been possible to produce uniformly a decline in blood pressure of 20 to 80 mm. in the hypertensive animals. Figure 3 illustrates the type of result from a single experiment with a series of six rats. Similar results have been obtained in several hundred experiments. Little or no decline in blood pressure has been noted in normal rats (fig. 4).

Most of the hypertensive rats had blood pressures ranging between 150 and 170 mm. of mercury before treatment and displayed no obvious changes in general condition when the blood pressure was reduced. Rats with marked hypertension—180 mm. of mercury or more—tolerated moderate decline in blood pressure without symptoms, but usually exhibited apathy, weakness and anorexia when the decline exceeded twenty-five or thirty millimeters. Still greater reduction frequently terminated in death—apparently from uremia. We assume that in these animals an adequate blood flow through the kidneys and other vital organs could be maintained only when the blood pressure was elevated, because secondary vascular changes had occurred as the result of the hypertension.

The degree of decline in blood pressure produced by a given amount of a given extract varied according to the initial level of the blood pressure, relatively greater diminution being observed in rats with higher initial pressures. When utilizing animals weighing 180 to 250 grams, one may define a rat unit as that amount of extract which causes reduction of 25, 35 and 50 mm. in rats with initial pressures of 150, 175 and 200 mm., respectively.

During the course of the experiments a number of the hypertensive rats became pregnant and we rather expected to find a sharp rise in the blood pressure during the latter part of gestation. To our surprise, each of the pregnant hypertensive rats showed, during the few days immediately before delivery, a well marked decline in blood pressure, the degree of reduction depending largely on the extent to which the blood pressure had previously been elevated. After parturition the blood pressure has slowly risen during a period of several days until the previous level was reached. Typical results are shown in figure 5. The decline in blood pressure has not been as striking as this in all the animals, but each pregnant hypertensive rat has displayed some reduction and a subsequent rise.

The explanation for these observations is not yet entirely clear. They

are, however, compatible with the assumption that in the rat the fetus produces some substance which is capable of causing a decline in the abnormally elevated blood pressure of the mother. The alternative explanation that the decline is due to an alteration in hemodynamic factors (*e.g.*, a decreased peripheral resistance) is improbable, when we consider the circulatory changes induced during pregnancy in the normal animal (8).

The elucidation of the apparent contradiction between the above described observations on rats and the frequent occurrence of hypertension in the so-called toxemias of pregnancy awaits further investigation.

BLOOD PRESSURE CHANGES IN PREGNANT HYPERTENSIVE RATS

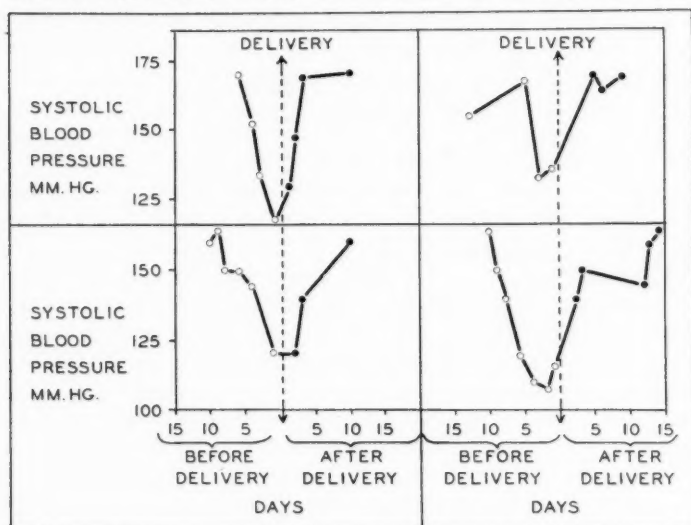


Fig. 5. In each rat a decline in blood pressure occurred during the latter part of pregnancy. Following parturition the pressure returned within a few days to the previous hypertensive level.

**DISCUSSION.** The findings which have been reported indicate clearly that extracts from the kidney may be prepared which in themselves have no significant effect on the blood pressure of normal rats, but which have the property of markedly reducing the pressor effect of certain substances when subsequently injected. For the sake of convenience the agent (or agents) responsible for this effect will be designated in the remainder of the discussion as the "*renal anti-pressor substance*." The discovery of the existence of such a substance will account for several phenomena which have been observed in regard to the pressor action of renal extracts.

Thus, Tigerstedt and Bergman and numerous subsequent observers, including ourselves, have found that crude extracts of renal tissue produce progressively smaller rises in blood pressure upon repeated injection. On the other hand more purified extracts have been shown by Pickering and Prinzmetal (7) to produce as great a response on repeated as on the initial injection. This discrepancy can be accounted for by the assumption that the cruder extracts contain the anti-pressor substance in addition to renin, while with purification the anti-pressor fraction is eliminated. The marked variability observed in the relation of the duration to the degree of the pressor response to crude preparations of renin (as shown in fig. 1) can also be attributed to differences in the relative proportions of renin and anti-pressor substance present in different preparations. The increase in sensitivity of rats to renin with increasing age (3) can also be explained if one makes the plausible assumption that the production of anti-pressor substance by the kidney decreases with advancing age.

It would be impossible to conclude solely on the basis of the observations which have been cited, that the anti-pressor substance is of physiological significance to the organism or is in any way concerned in the pathogenesis of renal hypertension. If, however, one accepts the view (which is as yet unproven) that some *renin-like* substance is the pressor agent responsible for renal hypertension, the demonstration that normal renal tissue contains an agent which is a physiological antagonist to it would serve to account for the known facts. Thus, the marked rise in blood pressure which follows the removal of the normal kidney from a dog with unilateral renal ischemia could be attributed to the elimination of the anti-pressor action of the normal kidney. The failure of hypertension to appear following bilateral nephrectomy can be explained by the fact that this procedure removes not only the anti-pressor agent but the pressor substance as well.

The available data can be interpreted as indicating either that the anti-pressor substance is a degradation product of kidney tissue which has a unique anti-pressor effect but fulfills no function in the normal animal, or that it is a specific substance of hormonal nature which normally protects the organism against hypertension and which cannot be elaborated in adequate quantities by the diseased kidney. Further work is in progress to settle these questions.

One point merits especial emphasis. Although objections may properly be raised against an anti-pressor effect obtained by parenteral administration as being due to noxious effects of toxic or depressor substances, such objections are scarcely applicable to those observations in which the anti-pressor action was elicited by the oral administration of extracts which in themselves produced no observable untoward effects and did not alter the blood pressure of normal animals.

## SUMMARY

Renal extracts were prepared which had no significant effects on the blood pressure when administered parenterally or orally to normal rats. The animals so treated exhibited, however, a well marked diminution in their pressor response to renin when compared to control animals which did not have preliminary treatment with the *pressor-free* renal extract. The agent responsible for this effect, which has in itself no direct action on blood pressure, has been designated as the "renal anti-pressor substance." The renal anti-pressor substance inhibits not only the response to renin but also that of ephedrin and possibly reduces also the pressor effect of pitressin.

Normal pregnant rats are less sensitive to renin than non-pregnant controls. Hypertensive rats display a pronounced spontaneous decline in blood pressure during the last few days of pregnancy, the blood pressure gradually returning, after delivery, to the previous level. These observations suggest that the fetus elaborates an anti-pressor substance.

Extracts containing the renal anti-pressor substance cause declines in the blood pressure of hypertensive rats when administered either parenterally or orally. Similar treatment does not alter the blood pressure of normal rats.

## REFERENCES

- (1) TIGERSTEDT, R. AND P. G. BERGMAN. *Skandinav. Arch. f. Physiol.* **8**: 223, 1898.
- (2) MERRILL, A., J. R. WILLIAMS, JR. AND T. R. HARRISON. *Am. J. Med. Sci.* **196**: 18, 1938.
- (3) GROSSMAN, E. B. AND J. R. WILLIAMS, JR. *Arch. Int. Med.* **62**: 799, 1938.
- (4) GROSSMAN, E. B. *Proc. Soc. Exper. Biol. and Med.* **39**: 40, 1938.
- (5) CHANUTIN, A. AND E. B. FERRIS, JR. *Arch. Int. Med.* **49**: 707, 1932.
- (6) WILLIAMS, J. R., JR., T. R. HARRISON AND A. GROLLMAN. *J. Clin. Investigation* **18**: 373, 1939.
- (7) PICKERING, G. W. AND M. PRINZMETAL. *Clin. Sci.* **3**: 211, 1938.
- (8) GROLLMAN, A. *The cardiac output of man in health and disease.* Charles C. Thomas, Springfield, 1932.

## GASTRIC CARBONIC ANHYDRASE IN DOGS

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Davenport and Fisher (1938) reported the discovery of large amounts of carbonic anhydrase in the gastric mucosa of cats, rats and rabbits. Davenport (1939) demonstrated that carbonic anhydrase is present in high concentration in the parietal cells and in lower concentration in the cells of the surface epithelium of the gastric mucosa of cats and rats. When those investigations were carried out dogs were not available.

At present work on the mechanism of the secretion of hydrochloric acid is being done using dogs as experimental animals. It is believed that carbon dioxide is formed in the parietal cells by the oxidation of a metabolite. This carbon dioxide is rapidly hydrated to carbonic acid with carbonic anhydrase catalysing the reaction. The carbonic acid then ionizes to give hydrogen ions and bicarbonate ions. By some means not at present understood the hydrogen ions are concentrated and secreted as the cations of hydrochloric acid while the bicarbonate ions are returned to the blood to replace the chloride ions removed in the acid secretion. Since the presence of carbonic anhydrase in the parietal cells is an important part of this hypothesis, it was thought desirable to demonstrate the occurrence of carbonic anhydrase in the gastric mucosa of dogs.

**METHODS.** The dogs were freed of blood by the viviperfusion method of Whipple (1926). The dogs were anesthetized with ether, and a cannula was inserted into the jugular vein. Ringer's solution containing 5 per cent glucose was injected by gravity, and the circulating fluid was allowed to flow out of the carotid artery at the same rate. By means of adrenalin injections the dogs' hearts were kept beating as long as possible. The hematocrit reading of the effluent fluid fell from about 38 per cent to below 1 per cent. At death the animals' tissues were not absolutely free of blood.

Carbonic anhydrase was determined by the method of Meldrum and Roughton (1933). The apparatus was used at 0°C. and at atmospheric pressure. The activity was calculated and expressed as enzyme units (E) according to the method of Meldrum and Roughton, but no correction was applied to bring the calculated activity to 15°C. The enzyme unit used in this work is therefore 2 to 3 times smaller than that of Meldrum and Roughton.

The distribution of carbonic anhydrase with respect to cell type was investigated by the method fully described by Davenport (1939). Cylinders 4.0 mm. in diameter were stamped from the gastric mucosa and placed on the table of a freezing microtome. The tissue was frozen, and sections 0.020 mm. in thickness were cut. Cutting was continued until *three consecutive sections* were obtained which were satisfactory. The 1st and 3rd slices were extracted with M/5 phosphate buffer, pH 6.8, and their carbonic anhydrase content was estimated.

The second slice was transferred to a microscope slide, fixed and stained. The slice was observed under a microscope, and the parietal cells in numerous fields taken over the whole slice were counted. Davenport (1939) has presented evidence to show that by this method a good estimate of the total number of parietal cells in the whole slice is obtained and that the number of cells found in the 2nd slice is equal to the mean of those in the 1st and 3rd slices.

**RESULTS.** Carbonic anhydrase was found in the extracts of the slices. The extracts catalysed the hydration and dehydration of carbon dioxide. The addition of the extracts to the phosphate buffer used in the enzyme estimation did not change the pH of the buffer as measured with a glass electrode. The end points of the catalysed and uncatalysed reactions were the same. The activity of the extracts was destroyed by 30 sec. boiling, by 30 min. at 65°C. and by 30 min. at pH 2 or 13. The activity was inhibited by M/800 HCN and by the specific inhibitor of Booth (1938).

The activity was probably not caused by the small amount of red blood cells remaining in the tissues. Dog red blood cells contain between 3.1 and 7.4 E per cmm. Up to 0.825 E was found in slices of the gastric mucosa having a volume of 0.25 cmm. If as much as 20 per cent of the volume of the slices consisted of perfusion fluid containing 1 per cent red blood cells the activity contributed by the carbonic anhydrase of the red blood cells would be only 0.0035 E per slice.

It was found that the slices fell into two distinct groups. The first group was composed of slices cut from the base of the glands, and they contained no surface cells. The second group was composed of slices cut from near the surface, and in addition to parietal cells and chief cells contained gastric pits made up of long tapering surface cells. The two groups will be considered separately.

For the first group the parietal cell count is plotted against the enzyme concentration in figure 1. The line drawn is the calculated regression line, and its equation is

$$Y = 0.000076 x + 0.023$$

where  $Y$  is the enzyme concentration per slice and  $x$  is the number of parietal cells per slice. The correlation coefficient ( $r$ ) calculated according

to the method of Fisher (1936) is  $+0.89$ , and there is less than one chance in a hundred that its true value lies outside the limits  $+0.54$  to  $+0.98$ . It is unlikely that any other type of cell would have a nearly perfect correlation with the parietal cells over the whole range of from no cells to 9000 cells per slice which makes it equally unlikely that the correlation between the parietal cells and the enzyme concentration is an artifact. The intercept on the Y axis is negligibly different from zero, so it is improbable that in these slices there is any significant amount of carbonic anhydrase in any other type of cell. Consequently it can be concluded that carbonic anhydrase is confined to the parietal cells.

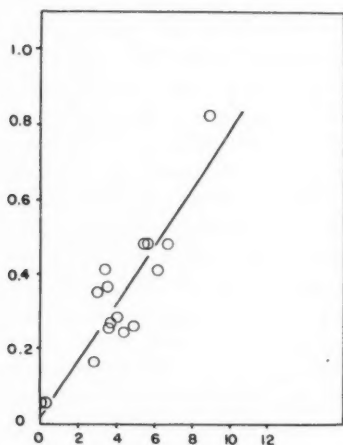


Fig. 1

Fig. 1. Abscissae: 1000 parietal cells per slice of dog gastric mucosa. Ordinate: Carbonic anhydrase in *E* per slice.

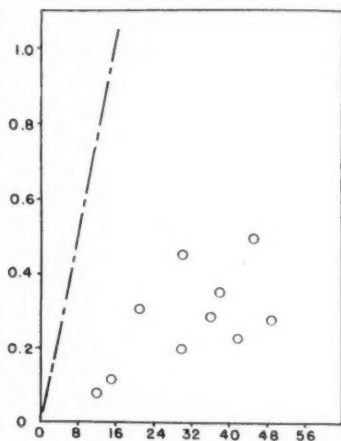


Fig. 2

Fig. 2. Abscissae: 1000 cells of the surface epithelium per slice. Ordinate: Residual carbonic anhydrase per slice. The broken line is the parietal cell regression line plotted on the same scale.

In the group containing surface cells the enzyme concentration was obviously not proportional to the number of parietal cells. The parietal cells were counted, and from the equation above the most probable amount of enzyme in that number of cells was calculated. This was subtracted from the total enzyme found. The surface cells were estimated, and their number is plotted in figure 2 against the residual enzyme concentration. The broken line is the parietal cell curve plotted on the same scale. Though the data are few and imperfect it can be concluded that there is probably a tenth as much carbonic anhydrase in the surface cells as in the parietal cells.

**CONCLUSIONS.** It has now been shown that there is a high concentration of carbonic anhydrase in the parietal cells of cats, rats and dogs. Since carbonic anhydrase has also been found in the gastric mucosa of several other mammals it is likely that parietal cells in all species contain carbonic anhydrase.

On account of such high concentration of carbonic anhydrase in the parietal cells the equilibrium between carbon dioxide and carbonic acid must be reached with extreme rapidity. The secretion of hydrochloric acid is doubtless a dynamic process, and during activity equilibrium is probably never quite attained. The observation of Bulger, Allen and Harrison (1928) that during acid secretion the bicarbonate content of the gastric venous blood rises leads to the conclusion that during activity carbon dioxide is continuously being produced within the cells and hydrated to carbonic acid and that the bicarbonate ions formed after the ionization of the carbonic acid are steadily flowing into the venous blood. The uncatalysed hydration of carbon dioxide is relatively slow, and if carbonic anhydrase were not present that reaction would be the limiting one in the chain. In fact the carbon dioxide would not be hydrated within the cells but would diffuse as such into the red blood cells where under the influence of the carbonic anhydrase present in the red blood cells it would be hydrated. Therefore the hydrogen ions produced by the subsequent ionization of the carbonic acid would be lost to the parietal cells and would instead be absorbed by the buffering mechanism of the blood.

The hypothesis that carbonic anhydrase occupies a central position in the mechanism of the formation and secretion of acid is further supported by unpublished observations that when carbonic anhydrase in the gastric mucosa is inhibited the secretion of acid is likewise inhibited to exactly the same degree as that to be expected if the rate of secretion is directly proportional to the rate of hydration of carbon dioxide. This work will be the subject of a paper in preparation.

#### SUMMARY

Carbonic anhydrase is present in high concentration in the parietal cells and in lower concentration in the cells of the surface epithelium of the gastric mucosa of the dog. It is probably not present in any other type of cell in the gastric mucosa.

#### REFERENCES

- BOOTH, V. H. *J. Physiol.* **91**: 474, 1938.  
BULGER, H. A., D. ALLEN AND L. B. HARRISON. *J. Clin. Investigation* **5**: 561, 1928.  
DAVENPORT, H. W. *J. Physiol.* **97**: 32, 1939.  
DAVENPORT, H. W. AND R. B. FISHER. *J. Physiol.* **94**: 16P, 1938.  
FISHER, R. A. *Statistical methods for research workers*. 6th ed., pp. 176-213, 1936.  
MELDRUM, N. U. AND F. J. W. ROUGHTON. *J. Physiol.* **80**: 113, 1933.  
WHIPPLE, G. H. *This Journal* **76**: 693, 1926.

## EFFECT OF CORTIN AFTER PARTIAL AND AFTER COMPLETE HEPATECTOMY

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In a previous communication we have shown that although cortin exerts no significant effect on the blood sugar under normal conditions, it may prevent both the hyperglycemia caused by adrenalin and the hypoglycemia elicited by insulin (1). It appeared, therefore, that the cortical hormone exerts a stabilizing action on the blood sugar under these conditions. It is well known, furthermore, that the marked decrease in the sugar and chloride concentration of the blood seen in adrenalectomized animals may be prevented by this hormone. The question arose whether this effect of cortin is specific and limited to the actions of adrenalin and insulin and to the prevention of the consequences of adrenal deprivation. A series of experiments has therefore been performed in which we studied the action of this hormone on the hypoglycemia and hypochloremia elicited by partial hepatectomy. We have previously shown that in the rat, removal of the median, right lateral and left lateral lobes of the liver (which represent approximately 85 per cent of the total liver weight) may easily be accomplished without damaging the remaining hepatic parenchyma. The resulting symptoms bear a striking resemblance to those of adrenal cortical insufficiency although rapid regeneration of the remaining liver tissue causes the changes to disappear within a few days (2). Among the changes produced by such partial hepatectomy, the most outstanding are the rapid decrease in the blood sugar and chloride content and in the blood volume and the formation of gastro-intestinal erosions. All of these changes are rather non-specific signs of general damage and may be produced by a great many noxious agents or severe surgical interventions. They are probably elicited by the serious metabolic disturbance caused by the sudden removal of the major part of the hepatic parenchyma. In previous communications, we have expressed the idea that the adrenal cortex plays an important rôle in combatting the effects of noxious agents and that the marked hypertrophy of the adrenal cortex which is seen following exposure to such agents represents an effort on the part of the organism to defend itself against non-specific damage (3) (4). This view has also been expressed in Leblond's recent review on the "alarm re-

action" (5). In the light of this hypothesis, one may argue therefore, that partial ablation of the liver causes a relative adrenal insufficiency since following this intervention, more than the normal amount of cortin is required. It seemed of particular interest, therefore, to establish whether the changes produced by partial hepatectomy can be prevented by cortin treatment.

**METHODS.** The partial hepatectomy has been performed by the method used by Selye et al. (2) which is a slight modification of that devised by Waelsch and Selye (6). A single ligature is placed around the common hilum of the median and left lateral lobes and another one around the hilum of the right lateral lobe of the liver. After the ligatures are tied, the lobes are removed and since in the rat, these three lobes always represent approximately 85 per cent of the total liver mass, one may be certain to have removed this same proportion of the liver tissue with a fair degree of accuracy in every case. Only in our first experiment (table 1) did we limit the operation to the removal of the median and left lateral lobes representing about 75 per cent of the liver tissue.

The blood sugar determinations were performed with the Somogyi modification of the Hartmann-Shaffer method. The chlorides were determined in whole blood by the Van Slyke method. Accurate measurements of the total blood volume have not been made but the animals were killed by cutting the jugular vein and carotid artery on one side and the escaping blood was measured directly. This does not give any data concerning the total amount of blood—as it does not take into account blood which may be stagnating in the portal circulation or the capillaries—but we found it an excellent index of the amount of freely circulating blood.

The cortical extract used was Wilson's preparation which contains one unit as defined by Selye and Schenker (7) in 0.03 cc.

In each experiment, the significance of the apparent differences between the cortin-treated and untreated series was evaluated by "Student's" method for small samples (8) and is expressed in the tables in terms of probability, estimated by graphic interpolation in Fisher's table of *t*. In accordance with the usual convention, differences between series cannot be accepted as significant when *P* is greater than 0.05. We are indebted to Prof. D. L. Thomson for these computations.

**EXPERIMENTAL RESULTS.** In our first experiment, 16 albino female rats weighing 94 to 112 grams were partially hepatectomized by removing only the median and left lateral lobes, and were then fasted for 24 hours. Food was withdrawn because we wanted to avoid any complication of the blood sugar values by post absorptive hyperglycemia and because previous experiments have shown that all signs of non-specific damage are more pronounced in fasted than in fed animals. During the 24 hours following partial hepatectomy, 8 of our rats received four subcutaneous

injections, each of 0.2 cc. of cortin and were killed by bleeding one hour after the last injection. The remaining animals acted as untreated partially hepatectomized controls. The average blood volume in the untreated partially hepatectomized animals was 1.86 cc. (range: 1.5-2.1), the average blood chlorides 425.8 (range: 410-456) and the average blood sugar 69.4 (range: 54-83). In the cortin treated group, the average blood volume was 1.825 cc. (range: 1.6-2.1,  $P = 0.72$ ), the average blood chlorides 443.1 (range: 421-467,  $P = 0.04$ ) and the average blood sugar 78.5 (range 63-87,  $P = 0.06$ ). From this it is evident that although both the blood chlorides and the blood sugar were higher in the treated than in the untreated series, only the former change proved statistically significant and the blood volume remained practically unchanged.

TABLE 1

|  | NUMBER OF ANIMALS |     |     |     |     |     |     | AVER-<br>AGE | P      |
|--|-------------------|-----|-----|-----|-----|-----|-----|--------------|--------|
|  | 1                 | 2   | 3   | 4   | 5   | 6   | 7   |              |        |
| Partially hepatectomized                     |                   |     |     |     |     |     |     |              |        |
| Blood volume (cc.)...                        | 1.7               | 3.0 | 2.0 | 0.8 | 1.3 | 2.2 | 0.6 | 1.66         |        |
| Chlorides (mgm. per<br>100 cc.).....         | 386               | 398 | 398 | *   | 392 | 392 | *   | 393.2        |        |
| Glucose (mgm. per<br>100 cc.).....           | 54                | 50  | 54  | 67  | 58  | 58  | 58  | 57.0         |        |
| Partially hepatectomized treated with cortin |                   |     |     |     |     |     |     |              |        |
| Blood volume (cc.)...                        | 4.2               | 3.0 | 3.2 | 0.6 | 1.5 | 2.2 | 2.4 | 2.44         | 0.19   |
| Chlorides (mgm. per<br>100 cc.).....         | 427               | 446 | 427 | *   | 415 | 410 | 415 | 423.3        | 0.0001 |
| Glucose (mgm. per<br>100 cc.).....           | 63                | 92  | 87  | 87  | 67  | 67  | 71  | 76.3         | 0.002  |

\* In these cases the blood volume was too low to allow of chloride determination.

More conclusive results have been obtained in a second experiment in which seven injections of 0.2 cc. of cortin were given to adult female albino rats, weighing 167 to 185 grams. In this and the following experiments, all three lobes (that is, 85 per cent of the liver) were removed and thus a more pronounced hypoglycemia was obtained in the controls not receiving cortin. Our results are summarized in table 1.

\* It will be seen that in this case, there was a marked difference between the two groups insofar as the decrease in blood chlorides and sugar was significantly inhibited by the cortin treatment. The average blood volume was lower after cortin treatment but not significantly, perhaps because in some animals more blood was lost during the surgical intervention.

In table 2 we give the results of an experiment in which adult male

albino rats weighing 170 to 323 grams were partially hepatectomized (three lobes removed) and then fasted for 24 hours, during which time half of them received two subcutaneous injections of 0.2 cc. of cortin and one injection of 0.4 cc., the last injection being given one hour before the animals were sacrificed. This amount was apparently not sufficient to prevent the fall in blood sugar, chlorides and blood volume completely, although some inhibition is noted here also.

Repeating this experiment on adult "hooded" females weighing 190 to 300 grams, we fasted the animals for 24 hours prior to the operation, then performed partial hepatectomy (three lobes removed) and administered 3 injections of 0.5 cc. at short intervals, one being given immediately after the operation, one 2 hours later and the last one, 3 hours later. The

TABLE 2

|  | NUMBER OF ANIMALS |     |     |     |     |     |     | AVER-<br>AGE | P     |
|--|-------------------|-----|-----|-----|-----|-----|-----|--------------|-------|
|  | 1                 | 2   | 3   | 4   | 5   | 6   | 7   |              |       |
| Partially hepatectomized                     |                   |     |     |     |     |     |     |              |       |
| Blood volume (cc.)....                       | 1.6               | 1.9 | 1.8 | 1.7 | 1.5 | 1.7 |     | 1.70         |       |
| Chlorides (mgm. per<br>100 cc.).....         | 351               | 374 | 363 | 363 | 386 | 374 |     | 368.5        |       |
| Glucose (mgm. per<br>100 cc.).....           | 39                | 58  | 31  | 39  | 43  | 31  |     | 40.1         |       |
| Partially hepatectomized treated with cortin |                   |     |     |     |     |     |     |              |       |
| Blood volume (cc.)....                       | 2.1               | 3.3 | 2.1 | 2.0 | 2.2 | 2.1 | 1.6 | 2.20         | 0.045 |
| Chlorides (mgm. per<br>100 cc.).....         | 386               | 398 | 351 | 380 | 363 | 409 | 386 | 381.9        | 0.19  |
| Glucose (mgm. per<br>100 cc.).....           | 67                | 43  | 62  | 43  | 71  | 62  | 58  | 58.0         | 0.011 |

animals were sacrificed one hour after the last injection. In this manner, the organism was flooded with cortical hormone during the relatively short period of the rapidly developing hypoglycemia caused by removal of 85 per cent of the liver in a previously fasted animal. In agreement with our expectations, the results were particularly striking in this group as shown in table 3.

From all these experiments, it seems evident that the decrease in blood volume (as estimated by our admittedly rough technique), the decrease in blood chlorides and the hypoglycemia caused by partial ablation of the liver are inhibited, though not completely prevented by suitable cortin therapy.

It seemed of interest to establish whether the hypoglycemia caused by complete hepatectomy could also be inhibited by the cortical hormone,

since new formation of sugar is not possible in the absence of the liver and some investigators expressed the view that cortin inhibits sugar utilization. If this be true, one should be able to delay the disappearance of circulating sugar after hepatectomy by cortin. This, however, is not the case. Table 4 shows the results obtained in a group of 12 adult albino male rats weighing 250 to 392 grams. In these animals, the liver was completely removed

TABLE 3

|  | NUMBER OF ANIMALS |     |     |     |     |     |     | AVER-<br>AGE | P     |
|--|-------------------|-----|-----|-----|-----|-----|-----|--------------|-------|
|  | 1                 | 2   | 3   | 4   | 5   | 6   | 7   |              |       |
| Partially hepatectomized                     |                   |     |     |     |     |     |     |              |       |
| Blood volume (cc.)...                        | 3.0               | 1.3 | 2.4 | 1.5 | 1.5 |     |     | 1.94         |       |
| Chlorides (mgm. per<br>100 cc.).....         | 392               | 386 | 386 | 380 | 392 |     |     | 387.2        |       |
| Glucose (mgm. per<br>100 cc.).....           | 58                | 39  | 31  | 58  | 43  |     |     | 45.8         |       |
| Partially hepatectomized treated with cortin |                   |     |     |     |     |     |     |              |       |
| Blood volume (cc.)...                        | 2.4               | 3.3 | 2.6 | 2.5 | 2.4 | 2.8 | 2.0 | 2.57         | 0.04  |
| Chlorides (mgm. per<br>100 cc.).....         | 409               | 409 | 392 | 386 | 427 | 427 | 443 | 413.3        | 0.023 |
| Glucose (mgm. per<br>100 cc.).....           | 75                | 62  | 79  | 75  | 58  | 71  | 71  | 70.1         | 0.001 |

TABLE 4

|   | NUMBER OF ANIMALS |     |     |     |     |     | AVERAGE | P    |
|---|-------------------|-----|-----|-----|-----|-----|---------|------|
|   | 1                 | 2   | 3   | 4   | 5   | 6   |         |      |
| Completely hepatectomized                     |                   |     |     |     |     |     |         |      |
| Chlorides (mgm. per 100 cc.).....             | 445               | 469 | 480 | 480 | 503 | 445 | 470.3   |      |
| Glucose (mgm. per 100 cc.).....               | 50                | 50  | 39  | 58  | 39  | 44  | 46.7    |      |
| Completely hepatectomized treated with cortin |                   |     |     |     |     |     |         |      |
| Chlorides (mgm. per 100 cc.).....             | 451               | 439 | 474 | 439 | 480 | 439 | 453.7   | 0.19 |
| Glucose (mgm. per 100 cc.).....               | 39                | 50  | 27  | 50  | 27  | 39  | 38.7    | 0.15 |

in one stage together with the alimentary tract, the animals were sacrificed one hour after the operation when signs of hypoglycemia began to be evident in some individuals. Half of these rats received 1 cc. of cortin subcutaneously immediately after the operation, while the other half remained untreated.

The blood sugar values were not significantly different in the two groups and the blood chlorides were actually above normal in the untreated

group, and at approximately the normal level in those treated with cortin. We do not know the reason for the increase in blood chlorides seen in this experiment, but it may possibly be due to the shorter time interval between operation and determination or to some disturbance in chloride metabolism caused by complete hepatic deficiency. In any case, this experimental arrangement did not seem suitable for the study of the hypochloremia preventing action of the cortical hormone. In repeating the experiment, we therefore, contented ourselves with blood sugar determinations to establish beyond doubt whether the hypoglycemia caused by complete hepatectomy remains uninfluenced by cortin. Twelve adult female albino rats weighing 160 to 205 grams were completely hepatectomized as in the previous series, half of them receiving 0.5 cc. of cortin subcutaneously, immediately after the operation and 0.2 cc. 30 minutes

TABLE 5

|   | NUMBER OF ANIMALS |     |     | AVERAGE |
|---|-------------------|-----|-----|---------|
|   | 1                 | 2   | 3   |         |
| Completely hepatectomized rabbits                     |                   |     |     |         |
| Glucose (mgm. per 100 cc.) before operation..         | 83                | 92  | 92  | 89      |
| 30 min. after operation .....                         | 92                | 132 | 75  | 100     |
| 60 min. after operation .....                         | 79                | 75  | 60  | 71      |
| 90 min. after operation .....                         | 83                | 62  | 27  | 57      |
| 120 min. after operation .....                        | 27                | 33  | 27  | 29      |
| Completely hepatectomized rabbits treated with cortin |                   |     |     |         |
| Glucose (mgm. per 100 cc.) before operation..         | 100               | 100 | 107 | 102     |
| 30 min. after operation .....                         | 100               | 115 | 96  | 104     |
| 60 min. after operation .....                         | 58                | 83  | 71  | 71      |
| 90 min. after operation .....                         | 50                | 83  | 27  | 53      |
| 120 min. after operation .....                        | 27                | 27  | 27  | 27      |

later. They were killed 90 minutes after the operation. The average blood sugar in the untreated group was 44 mgm. per cent (range: 39 to 45 mgm. per cent) and in the treated group, 46 mgm. per cent (range: 39 to 50 mgm. per cent).

We also repeated these experiments in the rabbit making blood sugar determinations 30, 60, 90 and 120 minutes after complete hepatectomy combined with removal of the gastro-intestinal tract. It was hoped that if there is only a slight and transitory inhibition of hypoglycemia by cortin, it would be easier to detect it on blood sugar curves. Table 5 indicates, however, that even under these conditions it is impossible to note any inhibitory influence, although the treated animals received 5 cc. of cortin intravenously immediately after the operation. The slight increase in blood sugar seen in a few animals 30 minutes after the intervention is possibly due to the handling of the liver during the operation.

It may be argued that the reason why cortin, though active in preventing the hypoglycemia caused by partial hepatectomy, has no effect on the decrease in blood sugar elicited by complete ablation of the liver, is simply that in the latter case, the fall is more rapid. We do not believe, however, that this explanation holds since the very rapidly developing insulin hypoglycemia is markedly inhibited by cortin as shown in our previous publication (1). It seems much more likely that cortin does not inhibit the utilization of circulating sugar but increases gluconeogenesis in the liver tissue and that it is, as a result of this, that it counteracts the hypoglycemia producing action of insulin and of the sudden removal of a large part of the hepatic glycogen stores.

#### SUMMARY AND CONCLUSIONS

Experiments on partially hepatectomized rats indicate that the decrease in the blood volume, blood chlorides and blood sugar caused by ablation of 85 per cent of the liver tissue in fasted rats is inhibited by administration of an adrenal cortical extract, rich in the life-preserving principle. Since the authors observed that cortin, though without significant effect on the normal blood sugar level, inhibits both insulin hypoglycemia and adrenalin hyperglycemia, they expressed the view that this hormone possesses a blood sugar stabilizing effect. The experiments presented in this communication give further support to this view.

The decrease in blood sugar produced by complete hepatectomy is not significantly influenced even by large doses of cortin. This finding makes it probable that the cortical hormone does not inhibit the utilization of circulating sugar. It seems more likely that it prevents the hypoglycemic action of insulin or the decrease in blood sugar following the removal of a large part of the hepatic glycogen stores by stimulating gluconeogenesis in the liver.

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#### REFERENCES

- (1) SELYE, H. AND C. DOSNE. *Proc. Soc. Exper. Biol. and Med.* **42**: 580, 1939.
- (2) SELYE, H., R. L. STEHLE AND J. B. COLLIP. *Canad. M. A. J.* **34**: 339, 1936.
- (3) SELYE, H. *This Journal* **122**: 347, 1938.
- (4) SELYE, H. *Arch. Internat. de Pharm.-dynamie et de Thérap.* **55**: 431, 1937.
- (5) LEBLOND, C. P. *Annales d'Endocrinol.* **1**: 179, 1939.
- (6) WAELSCH, H. UND H. SELYE. *Arch. f. exper. Path. u. Pharmacol.* **161**: 115, 1931.
- (7) SELYE, H. AND V. SCHENKER. *Proc. Soc. Exper. Biol. and Med.* **39**: 518, 1938.
- (8) FISHER, R. A. *Statistical methods for research workers.* 6th ed., p. 128 (Edinburgh, 1936).

## THE STANDARDIZATION OF TEMPERATURE REGULATORY RESPONSES OF DOGS TO COLD<sup>1</sup>

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For investigations of physiological temperature regulation it is necessary to have quantitative tests of the various components of the temperature regulatory system. The component functions which include shivering, peripheral vasomotor activity and panting (or sweating) must be evoked by a quantitative stimulus in a controlled environment and produce a response capable of quantitative measurement. In the experiments described in this paper these requirements have been fulfilled for shivering, vasoconstriction and vasodilatation produced by thermal stimuli. The "Regulationsbreite" test of Freund and Strassman (1912), in which the environmental temperature range is determined wherein a limited body temperature is maintained, is satisfactory for the integrated action of all component functions and is the ultimate test of homeothermy. This well known test, although extensively used, is not suitable for determining to what extent a component function is deficient in an experimental procedure. The tests here reported can be considered as supplementary to the Freund and Strassman procedure.

Trained unanesthetized short haired dogs weighing 10 to 15 kilos have been used exclusively. It has been found in agreement with Kitchen (1923) that dogs of the bull terrier type are most desirable due to their willingness to cooperate. As a quantitative measure of temperature regulating ability, skin and body temperatures at which vasomotor changes and shivering occur have been measured. Such an index is based on the fact that these responses occur as a result of changing temperatures of both skin and brain, a dual control as first proposed by Richet (1898) and now accepted by all physiologists. That a "peripheral control" of body temperature can occur was first demonstrated by Sihler (1879) who observed panting without a rise of rectal temperature in a dog when placed in the sunlight. Barbour (1912), Jelsma (1930), and Magoun, Harrison

<sup>1</sup> Technical assistance in this investigation was furnished by Onni Overhouse and George Cordes of the Works Progress Administration Official Project no. 665-71-3-69, Sub-Project no. 205.

and Brobeck (1938) have demonstrated beyond doubt the existence of "central control." With central regulation the responses mentioned can be elicited by varying the brain temperature without varying the skin temperature. Under practically all changing environmental conditions in which an animal maintains a normal temperature there is a simultaneous increase or decrease of both peripheral and central temperatures. Assuming the validity of Richet's theory, it is a variation of central or peripheral temperature or both which activates heating or cooling mechanisms. Hence both peripheral and rectal temperature thresholds for shivering and vasomotor responses have been measured.

As a means of applying a cold stimulus, resting in a cool, air conditioned room has been used. The three characteristics which define the cooling power of the environment, namely, 1, air temperature, 2, relative humidity, and 3, air velocity have been controlled. Environmental conditions have been determined in which the responses to cold of a trained dog are invoked without discomfort. In order to stop the cold responses or invoke the responses to warmth, heating by diathermy has been used as described in a previous paper (Hemingway, 1938). It has been shown previously (Hemingway and McClendon, 1933) that by using diathermy current of a frequency of one million cycles per second, a measured heat stimulus can be applied to a dog without any sensation other than that of heat. Furthermore, this heat stimulus has the advantage of being easily and accurately adjusted to any desired value. Such a control is possible because at a frequency of one million cycles per second and under diathermy conditions, current and voltage are in phase (Hemingway and McClendon, 1932), and the external heat production rate in the animal is simply high frequency voltage multiplied by the current.

Vasomotor activity as a response to heat or cold is conveniently measured by ear temperature variation (Grant, 1929-31; Grant and Bland, 1931-33). It has been shown in previous experiments (Hemingway, 1938), that the ear temperature of the dog is extremely labile. When ear vessels are dilated as a result of exposure to warmth, the ear temperature is 1 to 2 degrees below rectal and 1 to 3 degrees above trunk temperatures. When the ear vessels are constricted the ear temperature falls to values which are 7 to 10 degrees lower than trunk peripheral temperatures. In response to heat or cold, skin temperatures of the limbs vary between trunk and ear temperatures, but the response is sluggish in comparison to that of the ears. As a result of examining numerous temperature-time curves of skin and rectal temperatures it has been observed that the temperature of 30 degrees is in the interval where there is active constriction or dilatation of the ear vessels, as indicated by the most rapid change of ear temperature. For this reason in an animal being cooled the time when the falling ear temperature reaches 30 degrees is designated as the time of active vaso-

constriction. Similarly in an animal being warmed, active vasodilatation is to be considered as occurring when the ear temperature reaches 30 degrees. This is obvious from figure 1.

The determination of the onset of shivering by visual observation of muscular movements is unreliable for the slight movements of shivering which is just commencing. It has been found to be more satisfactory to use a shivering recorder as described below with which characteristic thermally induced shivering movements can be detected. With this recorder the time at which shivering commences can be accurately determined as well as various degrees of shivering intensity.

**EXPERIMENTAL. Vasomotor response.** In each test the following procedure was used. A normal short haired dog weighing 10-15 kilos and carefully chosen for resting in a basal state, was placed on his side on a shivering recorder, described below, in an insulated room with air temperature  $22.0 \pm 0.5$  degrees,  $50 \pm 5$  per cent humidity, and air velocity 25-40 feet per minute. The side of the animal which made contact with the recorder platform was shaved between the shoulder and hind leg. The shaved area rested on thin metal foil, which served as the "lower" diathermy electrode. An "upper" electrode was attached by elastic bands to the upper shaved surface of the leg. Thermocouples attached with adhesive tape  $20 \times 6$  mm. to the 1, upper ear; 2, anterior surface of foreleg; 3, exposed thorax; 4 and 5, skin under both electrodes recorded peripheral temperatures. In order to eliminate the high frequency thermocouple errors described by Huntoon (1937) the following precautions were taken. 1. The diathermy machine was equipped with appropriate choke coils. 2. The diathermy machine was placed on one side of the resting animal with the thermocouple-galvanometer system on the opposite side. 3. The bimetallic thermocouple junctions were covered with 5 thin layers of collodion. The high frequency errors can be determined by noting the change in deflection of the galvanometer as a result of turning on the current. The error was zero for ear, foreleg, thorax and rectum. When it did exist with temperatures of the skin beneath the electrodes the diathermy current was suspended for 15 seconds before the measurement was made. Temperatures were measured during the resting state every 1 to 3 minutes. When a steady state had been reached as judged by constancy of temperatures the diathermy current was turned on with a heat dosage equal to the dog's basal metabolic rate as determined by Kitchen (1923). Heating was continued until vasodilatation occurred as indicated by the sudden rise in ear temperatures.

**Shivering.** The onset of shivering was measured by the mechanical recorder shown in figure 2. The recorder consists of two adjacent rectangular platforms in the same horizontal plane which make contact at adjacent corners and are hinged together. One platform is stationary,

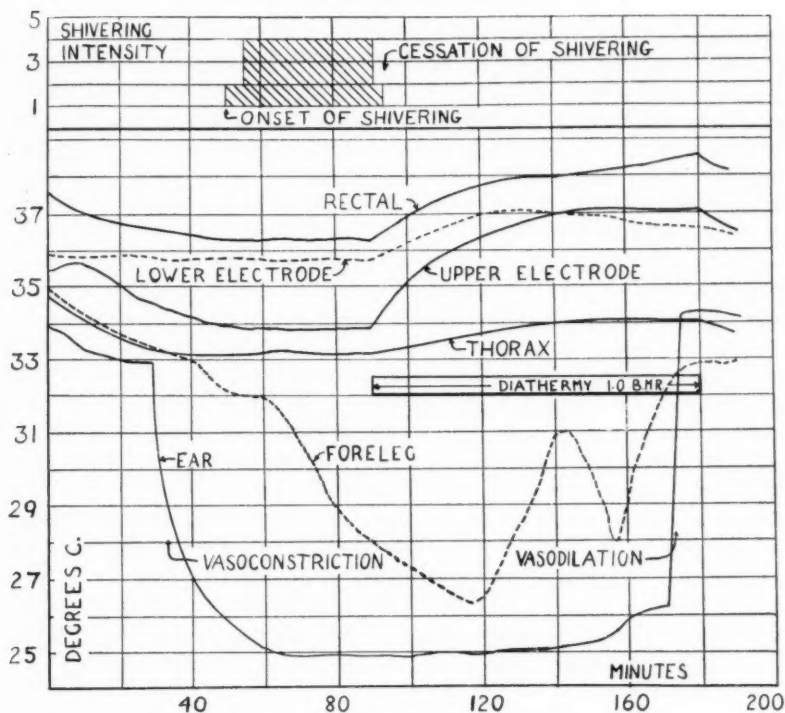


Fig. 1. Graphs of shivering and body temperatures plotted against time for a normal dog resting in a cool but not uncomfortable environment. Active vasoconstriction and vasodilation are occurring when the ear temperatures pass through 30 degrees.

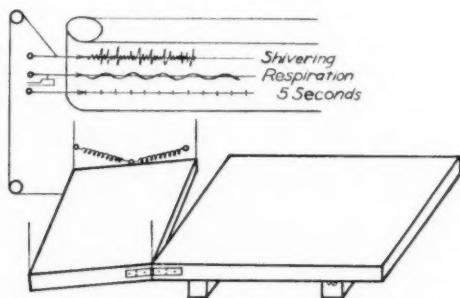


Fig. 2. Simplified diagram of a device for recording shivering and respiratory movements. The apparatus records mainly the characteristic abdominal flexion associated with incipient shivering.

resting on a table. The other platform is suspended by steel wires, 3 feet in length, from an iron frame, and rotates about the hinged corner in the horizontal plane. The forepart of the dog from the abdomen forward rested on the stationary platform while the hind legs and pelvis rested on movable platform. The hinged fulcrum is opposite the third lumbar segment of the resting dog so that abdominal flexion causes the movable platform to rotate toward the fixed platform. A damping spring limits the excursions of the movable platform. By means of thread, pulleys, and a lever a record of the movements of the platform can be obtained on a smoked kymograph drum on which is also registered respiratory movements from a chest cuff by means of a tambour. A resting dog gives only a barely perceptible respiration record from the moving platform. Shivering commences with an abdominal flexor twitch during inspiration. At the commencement of shivering there is a single abdominal flexion for every 5 to 10 inspirations. As shivering progresses these twitches increase in frequency until there is one for every inspiration. With continued cooling there develop several twitches with each inspiration and when shivering becomes intense there are twitches during all phases of the respiratory cycle. Shivering has been arbitrarily divided according to its intensity into the following five degrees of shivering, namely, 1, one twitch for more than 5 respirations; 2, one twitch for less than 5 respirations but more than one respiration; 3, one shivering twitch for each respiration; 4, 2 or more twitches during inspiration; 5, continuous twitching throughout the cycle. When shivering starts the first three degrees are passed through rapidly and a steady state is reached in stage 4 or 5. The shivering recorder was built after careful inspection of the shivering movements. It was designed especially to record the movement which was most characteristic of incipient shivering, namely, the abdominal flexion with an arching of the back.

**RESULTS.** For each test a graph similar to figure 1 was made in which temperature was plotted against time. Since reproduction of the curves for all of the experiments is not possible the most important data have been collected and are given in the tables. Four to 6 vasomotor-shivering tests were made on each of 4 selected dogs, an experiment lasting 3 to 5 hours.

*Vasomotor.* In table 1 are given the temperatures of foreleg, thorax, skin under electrodes, and rectum at which active vasoconstriction or vasodilatation occurred. Maximal, minimal, and average values are given for each set of experiments. At the foot of the table maximum and minimum and averages for all dogs are given. The vasoconstriction time is the time required for the ear temperature to fall from a value of 34 to 28 degrees. It is inversely proportional to the rate of vasoconstriction. The vasodilatation time is measured as the time interval in which the

temperature rises from 28 to 34 degrees during the diathermy treatment. The diathermy heating time for the vasomotor response is the time interval between the commencement of diathermy and active vasodilatation

TABLE 1  
*Vasomotor response*

|                           |        | COOLING BY RESTING IN A COOL ROOM |                 |         |      |        | VASOCONSTRICTION TIME | WARMING WITH DIATHERMY |                 |                 |         |     | VASODILATATION TIME | DIATHERMY TIME |
|---------------------------|--------|-----------------------------------|-----------------|---------|------|--------|-----------------------|------------------------|-----------------|-----------------|---------|-----|---------------------|----------------|
|                           |        | Degrees C.                        |                 |         |      |        |                       | Degrees C.             |                 |                 |         |     |                     |                |
| Rectal                    | Thorax | Upper electrode                   | Lower electrode | Foreleg |      | Rectal |                       | Thorax                 | Upper electrode | Lower electrode | Foreleg |     |                     |                |
| Dog A (5 experiments)     |        |                                   |                 |         |      |        |                       |                        |                 |                 |         |     |                     |                |
| Max.....                  | 37.6   | 35.0                              | 35.7            | 36.7    | 35.7 | 25     | 39.0                  | 34.8                   | 38.2            | 38.1            | 36.6    | 7.0 | 92                  |                |
| Min.....                  | 36.6   | 33.4                              | 34.0            | 35.4    | 32.0 | 7      | 37.7                  | 33.7                   | 34.0            | 36.1            | 25.3    | 1.0 | 31                  |                |
| Ave.....                  | 37.1   | 33.9                              | 34.5            | 36.0    | 33.9 | 14     | 38.4                  | 34.1                   | 36.1            | 37.1            | 31.5    | 3.0 | 68                  |                |
| Dog B (5 experiments)     |        |                                   |                 |         |      |        |                       |                        |                 |                 |         |     |                     |                |
| Max.....                  | 39.4   | 36.2                              | 36.5            | 36.2    | 31.9 | 26     | 40.1                  | 38.1                   | 39.0            | 39.1            | 26.7    | 3   | 103                 |                |
| Min.....                  | 38.5   | 34.6                              | 34.9            | 34.4    | 29.1 | 5      | 39.2                  | 35.2                   | 36.4            | 37.2            | 24.2    | 1.5 | 23                  |                |
| Ave.....                  | 38.8   | 35.4                              | 35.7            | 35.6    | 30.5 | 14     | 39.5                  | 35.9                   | 37.3            | 37.9            | 25.2    | 2.5 | 60                  |                |
| Dog C (4 experiments)     |        |                                   |                 |         |      |        |                       |                        |                 |                 |         |     |                     |                |
| Max.....                  | 39.2   | 35.1                              | 36.8            | 37.6    | 36.2 | 11     | 39.3                  | 35.3                   | 38.7            | 38.5            | 37.0    | 5.0 | 38                  |                |
| Min.....                  | 38.6   | 34.1                              | 35.7            | 36.0    | 34.6 | 9      | 38.8                  | 34.6                   | 36.4            | 37.4            | 28.7    | 1.0 | 13                  |                |
| Ave.....                  | 38.9   | 34.6                              | 36.1            | 37.1    | 35.5 | 10     | 39.1                  | 34.9                   | 37.9            | 38.1            | 33.9    | 3.2 | 21                  |                |
| Dog D (6 experiments)     |        |                                   |                 |         |      |        |                       |                        |                 |                 |         |     |                     |                |
| Max.....                  | 38.8   | 36.0                              | 36.8            | 37.8    | 36.4 | 23     | 39.5                  | 36.8                   | 38.4            | 38.6            | 38.4    | 5.0 | 68                  |                |
| Min.....                  | 38.1   | 34.0                              | 35.6            | 36.5    | 28.0 | 9      | 39.0                  | 34.1                   | 37.3            | 37.5            | 27.2    | 2.0 | 3                   |                |
| Ave.....                  | 38.4   | 35.4                              | 36.3            | 37.2    | 34.9 | 14     | 39.2                  | 35.7                   | 38.0            | 38.1            | 34.2    | 3.5 | 25                  |                |
| All dogs (20 experiments) |        |                                   |                 |         |      |        |                       |                        |                 |                 |         |     |                     |                |
| Max.....                  | 39.4   | 36.2                              | 36.8            | 37.8    | 36.4 | 26     | 40.1                  | 38.1                   | 39.0            | 39.1            | 38.4    | 7.0 | 103                 |                |
| Min.....                  | 37.1   | 33.4                              | 34.0            | 34.4    | 28.0 | 5      | 37.7                  | 33.7                   | 34.0            | 36.1            | 24.2    | 1.0 | 3                   |                |
| Ave.....                  | 38.3   | 34.8                              | 35.6            | 36.5    | 33.7 | 13     | 39.0                  | 35.1                   | 37.6            | 37.8            | 31.6    | 3.0 | 43                  |                |

indicated by the instant of transition of the rising ear temperature through 30 degrees.

*Shivering.* In table 2 are given the threshold temperatures at which shivering commenced while the animal was being cooled by resting in a cool room and the temperatures at which shivering ceased after com-

mencement of diathermy. The diathermy heating time for shivering is the interval between commencement of diathermy and cessation of shivering.

TABLE 2  
*Shivering response*

|                           | COOLING BY RESTING |            |        |         |      |                 |                 | WARMING BY DIATHERMY 1.0 B.M.R. |            |        |         |      |                 |                 |
|---------------------------|--------------------|------------|--------|---------|------|-----------------|-----------------|---------------------------------|------------|--------|---------|------|-----------------|-----------------|
|                           | Resting time       | Degrees C. |        |         |      |                 |                 | Diathermy time                  | Degrees C. |        |         |      |                 |                 |
|                           |                    | Rectal     | Thorax | Foreleg | Ear  | Upper electrode | Lower electrode |                                 | Rectal     | Thorax | Foreleg | Ear  | Upper electrode | Lower electrode |
| Dog A (5 experiments)     |                    |            |        |         |      |                 |                 |                                 |            |        |         |      |                 |                 |
|                           | min.               |            |        |         |      |                 |                 | min.                            |            |        |         |      |                 |                 |
| Max. ...                  | 70                 | 37.7       | 35.0   | 36.0    | 27.0 | 35.8            | 36.7            | 13                              | 38.3       | 34.6   | 36.5    | 25.2 | 38.4            | 38.2            |
| Min. ...                  | 35                 | 36.2       | 33.2   | 29.7    | 24.1 | 33.0            | 35.5            | 3                               | 36.8       | 33.6   | 26.3    | 23.2 | 33.5            | 35.4            |
| Ave. ....                 | 53                 | 36.9       | 33.7   | 32.3    | 24.8 | 34.0            | 36.0            | 8                               | 37.4       | 33.9   | 29.2    | 24.2 | 34.9            | 36.6            |
| Dog B (4 experiments)     |                    |            |        |         |      |                 |                 |                                 |            |        |         |      |                 |                 |
| Max. ...                  | 101                | 38.4       | 35.2   | 29.5    | 25.2 | 35.2            | 37.3            | 6                               | 38.7       | 35.9   | 27.0    | 24.9 | 35.4            | 37.7            |
| Min. ...                  | 60                 | 38.1       | 34.6   | 24.5    | 24.0 | 33.6            | 36.2            | 2                               | 38.4       | 35.0   | 24.0    | 23.6 | 34.4            | 36.5            |
| Ave. ....                 | 83                 | 38.2       | 34.9   | 27.1    | 24.3 | 34.5            | 36.7            | 4.5                             | 38.6       | 35.3   | 25.6    | 24.6 | 35.1            | 37.0            |
| Dog C (4 experiments)     |                    |            |        |         |      |                 |                 |                                 |            |        |         |      |                 |                 |
| Max. ...                  | 83                 | 38.8       | 34.8   | 35.8    | 27.5 | 36.0            | 37.6            | 6                               | 39.0       | 35.1   | 36.8    | 25.3 | 38.2            | 38.1            |
| Min. ...                  | 47                 | 38.4       | 33.9   | 33.0    | 25.0 | 35.6            | 37.0            | 3                               | 38.6       | 34.0   | 28.6    | 24.8 | 35.4            | 37.3            |
| Ave. ....                 | 68                 | 38.6       | 34.3   | 34.8    | 25.8 | 35.8            | 35.8            | 4                               | 38.8       | 34.7   | 34.0    | 25.0 | 37.0            | 37.8            |
| Dog D (5 experiments)     |                    |            |        |         |      |                 |                 |                                 |            |        |         |      |                 |                 |
| Max. ...                  | 125                | 38.4       | 35.9   | 36.6    | 34.5 | 36.8            | 37.8            | 5                               | 38.8       | 37.0   | 34.1    | 25.5 | 38.5            | 38.0            |
| Min. ...                  | 39                 | 38.2       | 34.9   | 35.6    | 25.5 | 34.3            | 36.2            | 1                               | 38.6       | 34.2   | 26.3    | 24.0 | 34.0            | 36.7            |
| Ave. ....                 | 71                 | 38.3       | 35.4   | 36.1    | 30.9 | 35.9            | 37.1            | 3                               | 38.7       | 35.6   | 30.6    | 24.7 | 36.9            | 37.5            |
| All dogs (18 experiments) |                    |            |        |         |      |                 |                 |                                 |            |        |         |      |                 |                 |
| Max. ...                  | 125                | 38.8       | 35.9   | 36.6    | 34.5 | 36.8            | 37.8            | 13                              | 39.0       | 37.0   | 36.8    | 25.5 | 38.5            | 38.2            |
| Min. ...                  | 35                 | 36.2       | 33.2   | 24.5    | 24.0 | 33.0            | 35.5            | 1                               | 36.8       | 33.6   | 24.0    | 23.6 | 33.5            | 35.4            |
| Ave. ....                 | 69                 | 38.0       | 34.6   | 32.6    | 26.5 | 35.1            | 36.4            | 5                               | 38.4       | 34.9   | 29.9    | 24.6 | 36.0            | 37.2            |

In table 3 are given the minimal rectal and thoracic skin temperatures of the dogs which were undergoing continuous shivering. The table also contains the resting time, which is the interval between the start of the experiment and the first appearance of these minimal temperatures. These three tables contain the pertinent information taken from twenty curves.

**DISCUSSION.** In the experimental procedures described above the requirements for quantitative stimuli in a controlled environment have been adequately fulfilled. The responses can be measured in the manner described in a quantitative way and the values in the table represent normal values. Any deviation from the normal response caused by an experimental process can be determined in a quantitative manner provided that the response falls outside the normal range of values.

The standardization of normal responses as described above is based on the results of previous work in which it has been shown that the temperature regulatory mechanisms can be motivated by 1, a change of peripheral

TABLE 3

*Minimal rectal and thoracic skin temperatures of resting shivering dogs and resting times until minimal temperatures were reached in a room of temperature  $22.0 \pm 0.5$  degrees, humidity  $50 \pm 5$  per cent and air velocity 25 to 40 feet per minute*

| DOG | NUMBER OF OBSERVATIONS | MINIMAL RECTAL TEMPERATURE | RESTING TIME | MINIMAL THORAX TEMPERATURE | RESTING TIME |         |
|-----|------------------------|----------------------------|--------------|----------------------------|--------------|---------|
|     |                        | $^{\circ}\text{C.}$        | <i>min.</i>  | $^{\circ}\text{C.}$        | <i>min.</i>  |         |
| A   | 5                      | 37.6                       | 65           | 35.6                       | 160          | Maximum |
|     |                        | 36.3                       | 38           | 33.1                       | 38           | Minimum |
|     |                        | 36.8                       | 51           | 33.9                       | 48           | Average |
| B   | 5                      | 38.7                       | 100          | 35.8                       | 90           | Maximum |
|     |                        | 38.1                       | 65           | 34.6                       | 20           | Minimum |
|     |                        | 38.2                       | 76           | 35.0                       | 73           | Average |
| C   | 4                      | 38.6                       | 70           | 34.5                       | 90           | Maximum |
|     |                        | 38.2                       | 40           | 33.3                       | 45           | Minimum |
|     |                        | 38.4                       | 55           | 34.1                       | 62           | Average |
| D   | 7                      | 38.6                       | 95           | 35.7                       | 105          | Maximum |
|     |                        | 38.0                       | 40           | 33.9                       | 55           | Minimum |
|     |                        | 38.3                       | 63           | 35.0                       | 71           | Average |

temperature, which stimulates skin receptors, and 2, a change of body or rectal temperature which stimulates a "center" in the hypothalamus. Just how much each contributes to the normal control of body temperature is not known since changing the external environment changes both skin and rectal temperatures. It is to be expected that both mechanisms function simultaneously, and for this reason both skin and rectal temperature changes were measured. The peripheral skin areas may be divided into three regions according to the temperature changes as a result of heating or cooling. These are (I) the trunk, head and neck; (II) ears and paws, and (III) legs. Most of the body surface is included on area I,

and this area shows the least temperature change after cooling or heating. The representative skin temperature of this region is the thoracic temperature. Area II shows the greatest temperature fluctuation, and area III has a variable response intermediate between the responses of areas I and II. In the case of reflex control motivated by peripheral thermal receptors, it is of interest to evaluate the importance by each of these areas. In the case of shivering the results show that impulses from cooled receptors of areas II and III have little or no effect for the following reasons: 1, shivering can be entirely suppressed during complete vasoconstriction of the ears, and 2, shivering in a few experiments commenced with a high ear temperature. This latter observation occurred only occasionally. One can conclude from these observations that it is trunk peripheral and rectal temperature that determines whether or not shivering starts or stops.

The sudden suppression of shivering after commencement of diathermy was striking. An animal would be undergoing violent shivering as a result of the prolonged rest. Within less than one minute after turning on the diathermy the shivering would diminish and after about 5 minutes would disappear completely. A similar effect in human beings has been observed by Uprus, Gaylor and Carmichael (1935). These investigators concluded that suppression of shivering was a central mechanism rather than peripheral, a fact which is in agreement with our observations but cannot as yet be accepted as conclusive (Jung, Doupe and Arnold, 1937).

The temperature-time curves were purposely drawn to observe the effects of rate of change of temperature. One would expect that a rapid fall in temperature of the skin or rectum would initiate shivering (or vasoconstriction) at a higher threshold temperature than a more slowly falling rate. However, the rates were extremely variable and no correlation was found between rates and thresholds. It is possible that the rates were all too low to act as stimuli. The conclusion which must be drawn is that threshold is a more important factor than rate of cooling in activating the shivering and vasomotor responses to cold.

There is a slight "lag" or "hysteresis" effect in that both vasomotor activity and shivering do not start or stop at one particular threshold temperature. Thus vasoconstriction occurs at a lower rectal and skin temperature than vasodilatation and a similar effect is observed for shivering. This is evident from the averages, which may be summarized as follows:

|                       | RECTAL | THORAX |                      |
|-----------------------|--------|--------|----------------------|
| Vasoconstriction..... | 38.3   | 34.8   | Falling temperatures |
| Vasodilatation.....   | 39.0   | 35.1   | Rising temperatures  |
| Shivering starts..... | 38.0   | 34.6   | Falling temperatures |
| Shivering stops.....  | 38.4   | 34.9   | Rising temperatures  |

There is a considerable variation among individual animals, e.g., animal A, a male bull dog of 12 kilos, had a temperature regulating mechanism which was set 1.0 to 1.8 degrees below that of the other dogs. A similar individual peculiarity was observed for panting dogs (Hemingway, 1938). The differences in thresholds of thoracic and rectal temperatures between different experiments on the same dog were usually within 0.5 to 0.7 degree, but an occasional abnormal threshold would appear giving the extreme variations of the tables.

It is to be noted that in general the temperature of the skin beneath the electrodes is 2 to 3 degrees above that of the exposed thorax while the diathermy current is on. Since they are temperatures of non-exposed skin areas they are 1 to 2 degrees above the thoracic temperature when no current is flowing. Heating by diathermy warms the skin as well as the body interior but the amount of heated skin is relatively small in comparison with other methods of raising body temperature, such as radiant heat, hot applicators or hot air due to the fact that diathermy heat is produced in the tissues rather than conducted in from the exterior.

The minimal temperatures in table 3 are the lowest which occur in a cooled animal under the conditions described. These low temperatures incite sufficient shivering and peripheral vasoconstriction to maintain a steady body temperature. These minimum temperatures were maintained at constant values once the steady state had been reached.

#### SUMMARY

Trained unanesthetized dogs have been slowly cooled by resting in a cool but not uncomfortable environment of  $22.0 \pm 0.5^{\circ}\text{C}.$ , humidity  $50 \pm 5$  per cent and an air velocity of 25 to 40 feet per minute. Respiratory rate and shivering movements have been measured together with rectal temperatures and skin temperatures on the thorax, foreleg, ear, and beneath two diathermy electrodes, one above and the other below the recumbent dog. After vasoconstriction and shivering had occurred the dogs were warmed by diathermy applied at a heating rate equal to the b.m.r. From the graphs, skin and rectal temperature thresholds were obtained for vasoconstriction, vasodilatation and shivering. These values represent normal responses to heat and cold in a controlled environment with a controlled heating rate, and are to be used as normal standards for comparison with responses when the temperature regulatory mechanism is impaired by drugs or lesions. A mechanical shivering recorder is described.

#### REFERENCES

- BARBOUR, H. G. Arch. f. exper. Path. u. Pharmacol. **70**: 1, 1912.  
FREUND, H. AND R. STRASSMAN. Arch. f. exper. Path. u. Pharmacol. **69**: 12, 1912.  
GRANT, R. T. Heart **15**: 281, 1929-31.  
GRANT, R. T. AND E. F. BLAND. Heart **16**: 69, 1931-33.

- HEMINGWAY, A. AND J. F. McCLENDON. This Journal **102**: 566, 1932.  
Physics **4**: 351, 1933.
- HEMINGWAY, A. This Journal **122**: 511, 1938.
- HUNTOON, R. D. Ann. Surg. **105**: 270, 1937.
- JELSMA, F. This Journal **93**: 661, 1930.
- JUNG, R., J. DOUPE AND E. ARNOLD. Brain **60**: 28, 1937.
- KITCHEN, H. D. This Journal **67**: 487, 1923.
- MAGOUN, H. W., F. HARRISON AND J. R. BROBECK. J. Neurol. **1**: 101, 1938.
- RICHET, C. Dictionnaire de Physiol. **3**: 175, 1898.
- SIHLER, C. J. Physiol. **2**: 191, 1879.
- UPRUS, V., G. B. GAYLOR AND E. A. CARMICHAEL. Brain **58**: 220, 1935.

## THE INFLUENCE OF POST-PITUITARY EXTRACT ON THE EXCRETION OF WATER AND CHLORIDES BY THE RENAL TUBULES

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It is generally agreed that the inhibition of water diuresis which follows the administration of post-pituitary extract is due to an increased re-absorption of water by the renal tubular epithelium (loop of Henle). No such agreement, however, has been reached concerning the mechanism of another phenomenon, the experimental evidence of which is equally well established. This is the fact that after the injection of post-pituitary extract, the output of urinary chlorides is increased, both relatively (per cubic centimeter of urine) and absolutely (per hour). Melville (1936) has interpreted this phenomenon on the basis of an increased "salt-mobilization" in the tissues. The experiments reported herein seem to indicate that this increased chloride excretion brought about by post-pituitary administration, like the decreased water excretion, is a renal and not a tissue phenomenon.

**METHODS.** Male white rats were used, weighing about 200 grams each. They were given in all cases a total of 10 cc. of fluid per 100 grams of body weight by intraperitoneal injection, and then placed in small wire-mesh metabolism cages set over aluminum funnels. The urine was collected in small graduated cylinders, measured, and analyzed for chlorides by the Volhard titration (Peters and Van Slyke, 1932) and for urea by the manometric hypobromite method (Van Slyke and Kugel, 1933). No preliminary fasting period was allowed; and the metabolic period was limited to 6 hours, this having been found to represent optimal conditions.

Besides distilled water, solutions containing 0.2, 0.4, 0.8, 1.0 and 1.2 per cent of sodium chloride were injected. In the series in which post-pituitary extract was used, this material was injected intraperitoneally 30 minutes before the administration of fluid and the start of the metabolic period.

At least twelve animals were tested with each strength of salt solution, with and without post-pituitary extract.<sup>1</sup> The dose of the extract rou-

<sup>1</sup> Posterior Pituitary Solution Squibb (U.S.P. XI strength), kindly furnished by Dr. John F. Anderson of E. R. Squibb and Sons.

tinely employed was 1.5 units per 100 grams of body weight, although a number of correlative observations were made using different dosages.

Similar experiments were also performed on a series of partially nephrectomized animals, in which at least 75 per cent of the renal tissue had been removed by a two-stage operation. The animals were used within the first month after operation, while body weight was still normally maintained and the animals were in apparent good health.

TABLE 1

*Effect of post-pituitary extract on the renal excretion of water, chlorides and urea after the administration of NaCl solutions\**

| SERIES                   | NUMBER OF EXPERIMENTS | NaCl INJECTED | Cl INTAKE | URINARY EXCRETION PER 100 GRAMS OF BODY WEIGHT PER 6 HOUR METABOLISM PERIOD (AVERAGE FIGURES $\pm$ PROBABLE ERRORS) |           |                |         |                |
|--------------------------|-----------------------|---------------|-----------|---|-----------|----------------|---------|----------------|
|                          |                       |               |           | Water   | Chlorides |                | Urea    |                |
|                          |                       |               |           |   | Per cc.   | Total          | Per cc. | Total          |
| Control                  |                       |               |           |   |           |                |         |                |
|                          |                       | per cent      | mgm.      | cc.   | mgm.      | mgm.           | mgm.    | mgm.           |
| A                        | 12                    | 0             | 0         | $6.2 \pm 0.3$   | 0.23      | $1.4 \pm 0.2$  | 3.20    | $19.8 \pm 0.9$ |
| B                        | 12                    | 0.2           | 12.2      | $6.2 \pm 0.2$   | 0.74      | $4.6 \pm 0.3$  | 3.80    | $23.5 \pm 1.4$ |
| C                        | 12                    | 0.4           | 24.4      | $5.0 \pm 0.2$   | 1.36      | $6.8 \pm 1.1$  | 4.02    | $20.1 \pm 0.6$ |
| D                        | 12                    | 0.8           | 48.7      | $3.5 \pm 0.2$   | 5.63      | $19.7 \pm 0.8$ | 7.17    | $25.1 \pm 1.6$ |
| E                        | 18                    | 1.0           | 60.9      | $2.3 \pm 0.2$   | 12.2      | $28.2 \pm 1.2$ | 9.30    | $21.4 \pm 0.8$ |
| F                        | 18                    | 1.2           | 73.1      | $2.0 \pm 0.1$   | 15.1      | $30.2 \pm 1.3$ | 14.0    | $28.0 \pm 0.7$ |
| Post-pituitary injected† |                       |               |           |   |           |                |         |                |
| G                        | 12                    | 0             | 0         | $0.8 \pm 0.07$  | 6.63      | $5.3 \pm 0.7$  |         |                |
| H                        | 12                    | 0.2           | 12.2      | $2.2 \pm 0.2$   | 9.45      | $20.8 \pm 0.7$ | 12.4    | $27.3 \pm 1.4$ |
| I                        | 12                    | 0.4           | 24.4      | $3.1 \pm 0.1$   | 9.80      | $30.4 \pm 1.1$ | 7.07    | $21.9 \pm 1.2$ |
| J                        | 12                    | 0.8           | 48.7      | $4.3 \pm 0.2$   | 10.3      | $44.5 \pm 1.1$ | 6.20    | $26.7 \pm 1.3$ |
| K                        | 12                    | 1.0           | 60.9      | $4.8 \pm 0.2$   | 10.6      | $51.0 \pm 1.2$ | 4.73    | $22.7 \pm 0.7$ |
| L                        | 12                    | 1.2           | 73.1      | $4.9 \pm 0.2$   | 11.3      | $55.5 \pm 2.1$ | 6.00    | $29.4 \pm 1.2$ |

\* All animals given a total of 10 cc. fluid per 100 grams of body weight.

† 1.5 units per 100 grams of body weight.

RESULTS. The results obtained from experiments on unoperated animals have been analyzed statistically and are shown in summary in table 1.

As the concentration of administered salt was increased, the inhibitory effect of the antidiuretic hormone on the excretion of water became less and less, and the urine volume increased over the control levels. The effect of extract administration on chloride excretion was to increase the urinary concentration of chlorides almost immediately to the "concentration ceiling," which in the rat is approximately 1 per cent. This

point was reached, under the influence of post-pituitary extract, after the administration of 0.2 per cent sodium chloride solution (table 1, H), whereas in contrast, in control animals, it required the injection of 1.0 per cent saline before similar urinary concentrations of chlorides were reached (table 1, E).

It will be noted that in every series the injection of extract brought about increased chloride excretion, both per cubic centimeter of urine and in total amount, as compared with the controls. Indeed, after the injection of 0.2 and 0.4 per cent sodium chloride, more chlorides were excreted than were originally administered (table 1, H and I), thus leading to body depletion.

By maintaining the chloride and water intake constant and varying the post-pituitary extract dosage or the amount of functional kidney tissue, it was possible to differentiate the changes in water excretion from those in salt excretion. In these experiments 0.2 per cent sodium chloride solution was routinely injected in order to maintain the level of chloride excretion below the "concentration ceiling" if possible.

After removal of about 75 per cent of the renal tissue in rats the remaining portion of the kidney hypertrophies (Chanutin and Ferris, 1932). This hypertrophy in our experiments was accompanied by progressive functional changes in which, apparently, the chloride-concentrating power was affected before any failure in the power to excrete injected water was evident. Thus, the surviving kidney tissue of partially nephrectomized rats was unable to concentrate chloride to the same extent as the controls, but still excreted water at normal rates (table 2, A compared with table 1, B). After the administration of post-pituitary extract to such animals, these changes were magnified: water excretion was inhibited to the same extent in both unoperated (table 1, H) and operated (table 2, B) animals, but the partially nephrectomized animals were relatively unable to concentrate chlorides.

Gradually reducing the dosage of post-pituitary extract also resulted in differentiating the salt from the water changes. The extract dosage was progressively halved from an initial dosage of 3.0 units per 100 grams of body weight. Over the dosage range from 3.0 units to 0.38 unit, the water excretion did not vary; but the chloride concentration of the excreted urine began to decline at 0.75 unit and fell sharply at 0.38 unit (table 3).

In contrast to the changes in chloride and water excretion following post-pituitary injection, which appear to be active processes associated with tubular reabsorption, the excretion of urea in these experiments was dependent only on the urine flow. The concentration of urea per cubic centimeter of urine was inversely proportional to the output of fluid, so that the total output of urea was constant. The grand average of all

urea determinations was  $24.2 \pm 0.36$  mgm. per 6 hour metabolic period, and no single series average differed significantly from this figure (table 1). The statistical identity of the curves of total urea excretion in both control and pituitary-injected animals indicates that the antidiuretic hormone is without direct influence on urea excretion.

TABLE 2  
*Water and chloride excretion in partially-nephrectomized animals receiving 0.2 per cent NaCl solution and post-pituitary extract\**

| SERIES                   | NUMBER<br>EXPERIMENTS | Cl INTAKE    | URINARY EXCRETION PER 100 GRAMS OF BODY<br>WEIGHT PER 6 HOUR METABOLISM PERIOD |              |             |
|--------------------------|-----------------------|--------------|--|--------------|-------------|
|                          |                       |              | Water  | Chlorides    |             |
|                          |                       |              |  | Per cc.      | Total       |
| Control (no extract)     |                       |              |  |              |             |
| A                        | 6                     | mgm.<br>12.2 | cc.<br>6.3   | mgm.<br>0.59 | mgm.<br>3.7 |
| Post-pituitary injected† |                       |              |  |              |             |
| B                        | 8                     | 12.2         | 2.3  | 5.44         | 12.5        |

\* All animals given a total of 10 cc. fluid per 100 grams of body weight.

† 1.5 units per 100 grams of body weight.

TABLE 3  
*Effect of different dosages of post-pituitary extract on water and chloride excretion of normal animals receiving 0.2 per cent NaCl solution\**

| SERIES | NUMBER OF EXPERIMENTS | Cl INTAKE | POST-PITUITARY EXTRACT INJECTED | URINARY EXCRETION PER 100 GRAMS OF BODY WEIGHT PER 6 HOUR METABOLISM PERIOD |           |       |
|--------|-----------------------|-----------|---------------------------------|---|-----------|-------|
|        |                       |           |                                 | Water   | Chlorides |       |
|        |                       |           |                                 |   | Per cc.   | Total |
|        |                       | mgm.      | units                           | cc.   | mgm.      | mgm.  |
| A      | 6                     | 12.2      | 3.0                             | 2.5   | 9.35      | 23.4  |
| B      | 12                    | 12.2      | 1.5                             | 2.2   | 9.45      | 20.8  |
| C      | 6                     | 12.2      | 0.75                            | 2.7   | 8.50      | 22.9  |
| D      | 6                     | 12.2      | 0.38                            | 2.7   | 6.05      | 16.3  |

\* All animals given a total of 10 cc. fluid per 100 grams of body weight.

DISCUSSION. The increased water excretion of post-pituitary-injected rats given 0.8 per cent or stronger salt solutions may be explained by the fact that once the "concentration ceiling" of approximately 1 per cent chloride is reached, the increased chloride clearance leads to increased water excretion simply by the osmotic binding of water. These results

confirm those reported by Nelson and Woods (1934) on white mice. In the present experiments the inhibitory effect of post-pituitary injection was overcome by the administration of 0.8 per cent sodium chloride solution; and the injection of post-pituitary extract along with more concentrated salt-solutions led to diuresis instead of inhibition.

Melville (1936) concluded that the augmentation of the diuretic effect of salts by post-pituitary extract, which he also observed in his experiments, was strong evidence that active salt mobilization occurs, which is not in any way related to the antidiuretic effect of the extract.

The experiments reported herein bear out completely the conception of the duality of post-pituitary response, i.e., the conclusion that the injection of the extract leads to two separate and distinct effects in the organism—the one concerned only with changes in salt metabolism, the other with water (Melville). But whereas Melville attributed the salt changes to a "salt-mobilizing" action in the tissues, the present experiments lead to the conclusion that the salt as well as the water changes are renal rather than extrarenal in origin. According to this concept, the antidiuretic hormone of the post-pituitary acts on the renal epithelium, not only to increase the reabsorption of water, but also to decrease the reabsorption of salt (chloride).

The evidence for this view may be summarized as follows: Under post-pituitary extract the chloride concentration per cubic centimeter of urine is increased faster than the urine volume decreases, an observation which may be explained either on the basis of decreased tubular reabsorption of chlorides or on the basis of increased glomerular filtration. Most investigators have concluded, however, that post-pituitary extract is without significant or consistent effect on the rate of glomerular filtration (Burgess, Harvey and Marshall, 1933; Peters, 1935; Smith, 1937), and the constancy of the urea excretion in these experiments permits the assumption that glomerular filtration is not significantly altered.

The results of experiments on partially nephrectomized rats are also pertinent. In these animals the concentration and excretion of injected salt is affected at a time when water is still normally excreted; and the simultaneous injection of post-pituitary extract renders the chloride-concentrating deficiency even more apparent. It may be assumed that at the time our experiments were performed only the renal tissues were affected, and the extrarenal tissues were still normal. Thus, if the antidiuretic hormone acted on the extrarenal tissues to release salt, such an effect should have been observed in partially nephrectomized as well as in normal animals. That it fails to act at the same time that the renal tubules begin to fail in their salt-concentrating function is an indication that the site of action of the extract is indeed renal.

It may further be noted that the injection of post-pituitary extract

into completely nephrectomized dogs failed to increase the blood chloride concentration (McIntyre and Van Dyke, 1931). This result is also in concordance with the hypothesis that the site of action of the hormone is the kidney rather than the tissues.

The theory of post-pituitary function set forth herein amplifies and extends the hypothesis of Silvette and Britton (1938) that "in the excretion of water and sodium chloride by the kidney, the diuretic hormone of the adrenal cortex acts in physiological antagonism to the antidiuretic hormone of the posterior lobe of the pituitary." The recent direct proof offered by Harrison and Darrow (1938) that one specific effect of lack of the cortico-adrenal hormone is the decrease in tubular reabsorption of sodium (chloride), taken in conjunction with the evidence offered herein, lends credence to the belief that the antagonism of the two principles has its seat of action in the tubular epithelium of the kidney.

#### SUMMARY

As the sodium chloride content of fluid injected into post-pituitary extract treated rats is increased, the urine volume progressively increases. The inhibitory effect of the extract was overcome by the administration of 0.8 per cent sodium chloride. At this point the "concentration ceiling" for chlorides was reached, and excess chlorides passing down the tubules carried with them additional amounts of water, osmotically held.

The injection of post-pituitary extract into rats given sodium chloride solutions ranging in concentration from 0 to 1.2 per cent led in all cases to increased chloride excretion, both per cubic centimeter of urine and in total amount, compared to control animals given similar solutions.

The concentration of urea per cubic centimeter of urine was inversely proportional to the urine output in both post-pituitary-injected and control series. The total amount of urea excreted during the metabolic period was constant, indicating that the extract is without direct influence on urea output.

As the dosage of post-pituitary extract was progressively reduced, the chloride-concentrating effect of the extract tended to disappear before the inhibitory effect on water excretion was affected.

In partially nephrectomized animals also the salt and water changes induced by post-pituitary extract were differentiated. In these animals the extract inhibited urine flow comparably as in the controls, but concentrated the chlorides to only about 50 per cent of the control levels.

From these and other considerations, the hypothesis is now advanced that the antidiuretic hormone of the post-pituitary gland acts directly on the kidney, leading to a decreased tubular reabsorption of salt (chloride) coincident with the well-known increased tubular reabsorption of water.

## REFERENCES

- BURGESS, W. W., A. M. HARVEY AND E. K. MARSHALL, JR. *J. Pharmacol. and Exper. Therap.* **49**: 237, 1933.
- CHANUTIN, A. AND E. B. FERRIS, JR. *J. Biol. Chem.* **49**: 767, 1932.
- HARRISON, H. E. AND D. C. DARROW. *J. Clin. Investigation* **17**: 504, 1938.
- MCINTYRE, A. R. AND H. B. VAN DYKE. *J. Pharmacol. and Exper. Therap.* **42**: 155, 1931.
- MELVILLE, K. I. *J. Physiol.* **87**: 129, 1936.
- NELSON, E. E. AND G. G. WOODS. *J. Pharmacol. and Exper. Therap.* **50**: 241, 1934.
- PETERS, J. P. *Body water: the exchange of fluids in man.* Springfield, 1935.
- PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry. Vol. II: Methods.* Baltimore, 1932.
- SILVETTE, H. AND S. W. BRITTON. *This Journal* **123**: 630, 1938.
- SMITH, H. W. *The physiology of the kidney.* New York, 1937.
- VAN SLYKE, D. D. AND V. H. KUGEL. *J. Biol. Chem.* **102**: 489, 1933.

EFFECT OF ABLATING THE FRONTAL LOBES, HIPPOCAMPUS,  
AND OCCIPITO-PARIETO-TEMPORAL (EXCEPTING PYRIFORM AREAS) LOBES ON POSITIVE AND NEGATIVE OLFACTORY CONDITIONED REFLEXES

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A mode of acquiring positive and negative olfactory conditioned reflexes<sup>1</sup> together with the effects of transecting the fornices and extirpating the motor cortex from one and both sides has been reported previously. This investigation is concerned with the effects of the ablations listed in the title on acquired and on ability to acquire olfactory conditioned reflexes. All animals were subjected to the following tests: *a*, establishment of a conditioned foreleg response to clove vapor; *b*, ability to transfer this reflex to the opposite foreleg; *c*, ability to establish a negative foreleg response to asafetida or the differentiation between two olfactory conditioned reflexes, which means that the dog must decide in 7 seconds whether to jerk his foreleg for cloves and not jerk it for asafetida; *d*, ability when blindfolded to find a certain pan and select a paper package containing meat from 3 paper packages of like size and texture.

*Procedure.* The manner of excluding light and sound, the method of inhalation, the use of empty bottle controls and the apparatus used for recording has been described in earlier papers. As for anatomical controls—careful observations were made of the damage to the brains both at

<sup>1</sup> In acquiring a negative conditioned foreleg response to asafetida which involved not only a discrimination between cloves and asafetida but a positive or negative response in 7 seconds it was important: 1, that the clove reflex is established perfectly for one foreleg. 2, that each asafetida test be preceded and followed by several clove tests, the usual ratio being 5 cloves to 1 asafetida, but it was advantageous to change this ratio in some animals and in all animals occasionally to eliminate the possibility of forming a sequence reflex. 3, that if the dog persists in responding positively to asafetida with the usual procedure he can usually be trained not to respond by a slap with a strap. 4, that the temperament of the animal should be understood for dogs differ as much as people. The dogs should be young and alert and not too irritable or too docile. If supersensitive to shock the dog may give frequent anticipatory responses, ready to pull at any signal or may hold up leg for long intervals of time. These conditions were corrected by rest, by whipping and by attaching a lead weight to foreleg. Bull dogs would frequently not coöperate and females were often of the martyr type.

autopsy and from formalin preparations. Marchi sections were made of the medulla and of the hypothalamus to determine the amount of degeneration in the pyramids and fornices.

*Operations.* The frontal approach was used for all lesions excepting the frontal lobes which were removed through the frontal sinuses. All of the general cortex ablations were first circumscribed by a chisel possessing a depth gauge and removed in one piece by a spatula. After it had been shown that large areas of the parietal, occipital and temporal (exclusive of the pyriform-amygdaloid complex) lobes were not essential for establishment of positive and negative olfactory conditioned reflexes, the hippocampi, dentate gyri and fornices were exposed with little bleeding by making a long slit through the gyrus suprasylvius into the ventricle with an electric cutting knife and elevating the cephalic margin of the gyrus with a spatula. These structures were then either extirpated after drawing a small semi-circular knife under (median) them in a vento-dorsal direction or coagulated with a series of burns made with the ball electrode of a surgical unit.

**PARIETO-OCCIPITO-TEMPORAL ABLATIONS.** It seldom happened that the hippocampi or pyriform lobes were involved in these lesions, but in some instances when the incisions extended some distance into the gyrus coronalis, sufficient cortico-spinal fibers were injured to cause paralysis of the opposite limbs.

*Effect on dogs previously conditioned.* The extent of these bilateral extirpations (fig. 1 A to C) included the greater part of the above mentioned lobes (exclusive of the pyriform areas and hippocampi). Except for dog 3 paralyzed in left foreleg, these dogs behaved as normal dogs. Marchi sections through the hypothalamus and medulla revealed no more than normal amount of degeneration in the fornices or in any of the pyramids with the exception of the right pyramid in dog 3, which was filled with degenerated sheaths.

Considerable data from many series of 25 tests taken before and after operating are collected in table 1. It is clear from columns 1 to 6 of this table that these three dogs experienced no difficulty in acquiring the positive clove response in the usual number of trials (25) or transferring this reflex from one foreleg to the other or establishing a negative response to asafetida. The 7th and 8th columns show that the cortical extirpations in no way interfered with the clove reflex previously acquired. Columns 10 and 11 show that the extirpations produced no diminishment in the ability of these dogs to transfer the clove reflex from one foreleg to the other. Columns 9 and 12 likewise reveal no loss of ability to respond correctly to cloves and asafetida. The ratio of asafetida negatives to clove positives was fully as great as before the ablations were made and the first trial of asafetida was negative for each dog.

*Effect of lesions on conditioning.* The extent of the cortical ablations in this group of dogs is shown in figure 1 (*E* to *I*); from which it is obvious that the greater part of the parietal, occipital and temporal (exclusive of the pyriform area and hippocampus) cortex was removed from dogs 5 and 7 (*E* and *G*) and slightly lesser amounts from dogs 6, 8 and 9 (*F*, *H* and *I*).

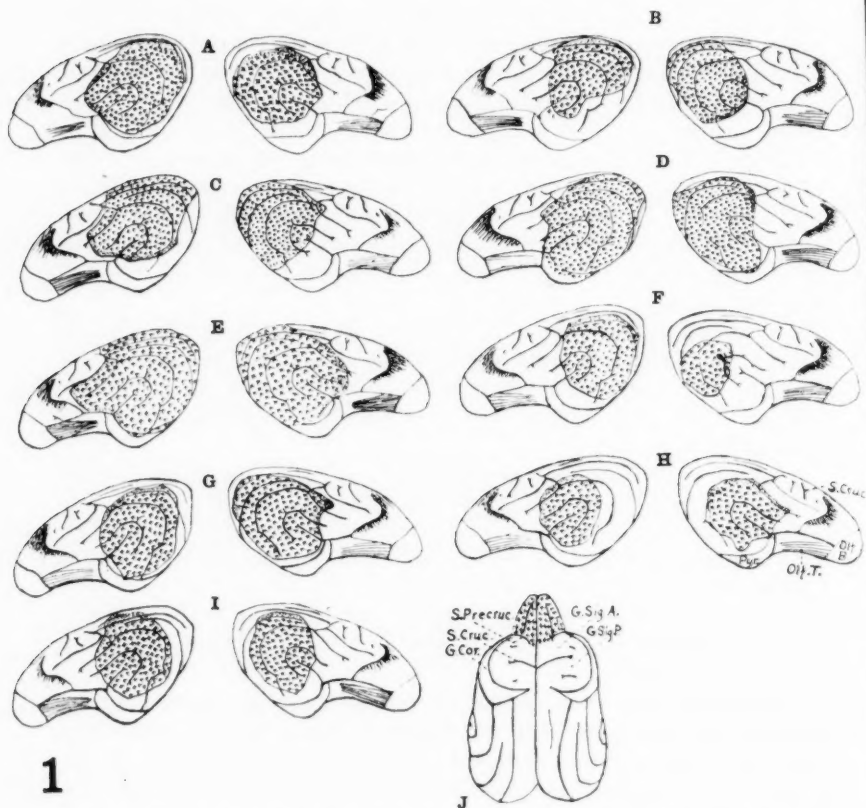


Fig. 1. *A* to *I* show the extent of the bilateral occipito-parieto-temporal extirpations and *J* a typical ablation of the frontal lobes. Crosses indicate that damage extended to the white matter and dots that varying portions of the cortex were injured.

As a result of these extensive lesions dogs 5, 7 and 9 were paralyzed and did not place with one foreleg. Marchi sections through the medulla and hypothalamus revealed the pyramids opposite of the paralysis to be full of degenerated sheaths and no more than normal degeneration in the opposite pyramids or in the fornices.

Columns 1 and 2 of table 2 reveal the establishment of the positive clove reflex in dogs 5, 6 and 7 with no more delay than in unoperated dogs. In fact the records of dogs 5 and especially 7 have been equaled by very few normal dogs. The delay in dogs 8 and 9 can be explained by timidity of the martyr type in 8 and paralysis in 9.<sup>2</sup> Concerning the differential tests the third and sixth columns of table 2 disclose that a negative response to asafetida was as readily acquired as in an unoperated dog. The remaining columns of this table show no increased difficulty of these dogs to transfer these reflexes from one foreleg to the other.

TABLE 1

*Effect of large parieto-occipito-temporal ablations on acquired olfactory conditioned reflexes*

| DOG<br>NUM-<br>BER | BEFORE EXTIRPATION                                 |   |   |                              |                          |  | AFTER EXTIRPATION            |                          |  |                              |                          |  |
|--------------------|--|---|---|------------------------------|--------------------------|--|------------------------------|--------------------------|--|------------------------------|--------------------------|--|
|                    | First foreleg                                      |   |   | Second foreleg               |                          |  | First foreleg                |                          |  | Second foreleg               |                          |  |
|                    | First genuine clove response came with test number | Clove reflex established with test number | Negative response to asafetida established with test number | First genuine clove response | Clove reflex established | Negative response to asafetida established | First genuine clove response | Clove reflex established | Negative response to asafetida established | First genuine clove response | Clove reflex established | Negative response to asafetida established |
| 1                  | 11<br>R  | 15  | 2   | 18<br>L                      | 20                       | 1  | 7<br>R                       | 8                        | 1  | 1<br>L                       | 2                        | 1  |
| 2                  | 8<br>R   | 24  | 1   |                              |                          |  | 2<br>R                       | 4                        | 1  | 23<br>L                      | 24                       | 1  |
| 3                  | 2<br>R   | 3   |   | 9<br>L                       | 10                       | 6  | 78*<br>L                     | 83                       | 1  | 4<br>R                       | 5                        | 1  |

R or L denotes order in which right and left legs were tested.

\* Signifies left foreleg was paralyzed after operation.

In one dog the effects of unilateral and bilateral ablations shown in figure 1 *D* were tested with the result that they were comparable in every way to a normal dog.

All of the previously mentioned cortical extirpated dogs after having learned that a paper package might contain meat, were able when blind-folded to select instantly and open a paper package containing meat when

<sup>2</sup> It is of interest to record that this dog showed the same anticipatory responses with the paralyzed leg during the clove tests as were characteristic for previously described dogs with delayed motor cortex. These responses consisted of escape movements and crying or the continuous lifting of one leg or balking on route from the animal quarters to laboratory. The escape movements and crying were most pronounced during and between the clove tests, but never occurred during the asafetida tests. This vapor had a very quieting effect.

placed in a pan with 3 paper packets of like size and texture. Their taste responses were also the same as normal dogs to solutions of sugar, salt, quinine and acetic acid dropped on their tongues.

TABLE 2  
*Effect of large parieto-occipito-temporal ablations on acquiring olfactory conditioned reflexes*

| DOG<br>NUM-<br>BER | FIRST FORELEG   |   |  | SECOND FORELEG                  |                             |  | SECOND TRANSFER                 |                             | THIRD<br>TRANSFER           |
|--------------------|---|---|--|---------------------------------|-----------------------------|--|---------------------------------|-----------------------------|-----------------------------|
|                    | First genuine<br>clove response<br>came with test<br>number | Clove reflex<br>established with<br>test number | Negative response<br>to asafetida<br>established with<br>test number | First genuine<br>clove response | Clove reflex<br>established | Negative response<br>to asafetida<br>established | First genuine<br>clove response | Clove reflex<br>established | Clove reflex<br>established |
| 5                  | 6<br>L  | 9   | 6  | 26*<br>R                        | 190                         | 1  | 4<br>L                          | 5                           | 1<br>R                      |
| 6                  | 16<br>R   | 31  | 1  | 3<br>L                          | 11                          | 1  | 29<br>R                         | 32                          | 1<br>L                      |
| 7                  | 2<br>R  | 3   | 1  | 15*<br>L                        | 30                          | 1  | 3<br>R                          | 4                           | 1<br>L                      |
| 8                  | 74<br>R   | 77  | 1  | 3<br>L                          | 11                          | 2  | 2<br>R                          | 3                           |                             |
| 9                  | 101*<br>R   | 105   | 2  | 2<br>L                          | 6                           | 1  |                                 |                             |                             |

R or L denotes order in which right and left legs were tested.

\* Signifies that a foreleg was paralyzed from operation.

EFFECT OF EXTIRPATING BOTH HIPPOCAMPI (AMMONS' HORNS). Since there is a possibility that all of the efferent fibers from the hippocampus do not go to the diencephalon by way of the fornix, but may go to the cerebral cortex, it seemed desirable to supplement earlier work in which the fornices were cut with experiments in which both hippocampi were ablated.

*Conditioning followed removal.* Data from many series of clove and differential tests from 5 dogs in which the hippocampi were removed by the knife method are summarized in table 3. The first two columns of this table show that the ablations produced no delay in the appearance of the clove response for dogs 11, 12 and 14 and but little, if any, delay in dogs 10 and 13. Column 3 reveals that none of these dogs required more than 2 differential trials to establish a negative response for asafetida, which

is a better average than the ordinary run of normal dogs would give. Columns 4 and 5 disclose that eliminating the hippocampi did not interfere with the transference of the clove reflex from one foreleg to the other, provided due allowance is made for paralysis in dogs 10 and 14. Column 6 demonstrates that these dogs with deleted hippocampi could also respond correctly to cloves and asafetida with the opposite forelegs.

The following observations were recorded at autopsy and from dissections of formalin prepared brains from this group of dogs: The hippocampi were completely removed from the left side of dog 10 and from the right

TABLE 3

*Effect of removing both hippocampi (Ammon's horns) on acquiring olfactory conditioned reflexes*

| DOG | FIRST FORELEG                                      |   |   | SECOND FORELEG               |                          |  |
|-----|--|---|---|------------------------------|--------------------------|--|
|     | First genuine clove response came with test number | Clove reflex established with test number | Negative response to asafetida established with test number | First genuine clove response | Clove reflex established | Negative response established to asafetida |
| 10  | 32<br>L  | 42  | 2   | 78*<br>R                     | 102                      | 1  |
| 11  | 5<br>R   | 8   | 1   | 18<br>L                      | 19                       | 2  |
| 12  | 13<br>R  | 14  | 2   | 11<br>L                      | 12                       |  |
| 13  | 35<br>R  | 63  | 1   | 19<br>L                      | 20                       | 1  |
| 14  | 15<br>L  | 16  | 1   | 30*<br>R                     | 43                       | 1  |

R or L denotes order in which forelegs were tested.

\* Signifies that a foreleg was paralyzed from operation.

side of dogs 11, 12, 13 and 14. Remnants of 5 to 6 mm. of the extreme ventral ends of the opposite hippocampi persisted in dogs 10, 11, 12 and 14. In dogs 10, 11 and 14 this piece was yellow in color and histological sections indicated incapability of functioning, but in dog 12 it appeared normal. In dog 13 the ventral remnant was longer, about 10 mm., the dorsal portion appearing abnormal and the ventral normal. There was apparently no damage to the pyriform-amygdaloid complex in any of these dogs. Marchi sections of the medulla revealed complete degeneration of the fiber sheaths of the left pyramid in dogs 10 and 14 and about half of the sheaths of the left pyramid of dog 12, but with no more than normal degeneration in the

opposite pyramids of these dogs or in both pyramids of dogs 11 and 13. Marchi sections through the hypothalamus showed the fornices of all these dogs to be filled with degenerated sheaths.

*Conditioning preceded and followed removal.* Dog 15 which can be taken as a type for the other dogs of this group acquired the first clove response during the 4th trial and the reflex became well-established after the 16th. Differentiation between cloves and asafetida came with the first series of tests. After operating, 45 clove and 9 asafetida tests resulted in all but one clove test being positive in 1 to 3 seconds and all of the asafetida trials were negative for at least 7 seconds.

Dogs 16 and 17 after acquiring the clove reflex within 25 trials experienced some difficulty in differentiating between cloves and asafetida so that the whipping technique was used to hasten the result. Both dogs were able to respond correctly to cloves and asafetida before operating and after removing the hippocampus from one and both sides. It should be recorded that many more differential tests were made with these dogs than with dog 15.

Dissection of the brain of dog 15 showed both hippocampi gone except for about 7 mm. of their extreme ventral tips and the dorsal half of these fragments had a distinct yellow color. None of the left hippocampus was present in dogs 16 and 17; but about 10 mm. of the extreme ventral portion of the right hippocampus remained, and while of good color ventrally, histological sections indicated incapability of functioning. Marchi sections through the hypothalamus portrayed marked degeneration in the fornices of all the dogs and the left pyramid of dog 17 contained many degenerated fibers.

For all the dogs with deleted hippocampi we would not concede, as a maximum, more than 5 mm. (about 12 per cent of the total structure) of any hippocampus capable of functioning. For some of these dogs it is very doubtful if any part of either hippocampus functioned after ablation.

Three dogs in which the hippocampi were completely destroyed by coagulation together with some damage to the pyriform lobes and amygdaloid nuclei demonstrated ability to acquire the clove reflex, but very little ability to respond correctly in 7 seconds to cloves and asafetida. For the present it seems best to attribute this interference to pyriform-amygdaloid damage. Unfortunately these tests were made before the whipping technique had been used to punish positive responses for asafetida.

After having learned that a paper packet might contain meat, all of the dogs with hippocampi removed were able immediately to select a paper package containing meat when placed in a pan with 3 paper packages of like size and texture. Also their taste responses to sugar, salt, quinine and acetic acid were not altered.

**FRONTAL LOBECTOMY.** In these extirpations it was essential to remove

these lobes without injury to the motor cortex, the cortico-spinal fibers or the olfactory tracts and bulbs. The first two were avoided by keeping rostral to the precruciate sulcus and directing the spatula slightly forward upon insertion. There is little danger of injuring the olfactory tracts and bulbs due to their extreme ventral position in the dog. Upon exposure, the openings of the frontal sinuses into the nasal passages were closed with swabs until the lesion was completed. Sinus infections were rare and those which occurred were confined to their immediate region. In some instances sufficient edema accumulated to require draining.

*Ablations preceded conditioning.* The second column of table 4 clearly demonstrates an observation that was very noticeable in these tests,

TABLE 4  
*Effect of frontal lobectomy on acquiring olfactory conditioned reflexes*

| DOG | FIRST FORELEG                                      |   |                                    |                                    |   |   | SECOND FORELEG               |                          |                                    |                                    |   |   |                |
|-----|--|---|------------------------------------|------------------------------------|---|---|------------------------------|--------------------------|------------------------------------|------------------------------------|---|---|----------------|
|     | First genuine clove response came with test number | Clove reflex established with test number | Differentiation tests              |                                    |   |   | First genuine clove response | Clove reflex established | Differentiation tests              |                                    |   |   | Differentiates |
|     |  |   | Number of clove responses positive | Number of clove responses negative | Number of asafoetida responses positive | Number of asafoetida responses negative |                              |                          | Number of clove responses positive | Number of clove responses negative | Number of asafoetida responses positive | Number of asafoetida responses negative |                |
| 19  | 3  | 76  | 303                                | 30                                 | 63                                      | 4                                       | 9                            | 15                       | 149                                | 5                                  | 29                                      | 1                                       | No             |
| 20  | 21   | 277                                       | 261                                | 64                                 | 60                                      | 5                                       | 18                           | 41                       | †                                  |                                    |   |   | No             |
| 21  | 37   | 65  | 248                                | 52                                 | 55                                      | 5                                       | 3                            | 4                        | 171                                | 4                                  | 34                                      | 1                                       | No             |
| 22  | 45   | 206                                       | 39                                 | 11                                 | 9                                       | 2                                       | *                            |                          |                                    |                                    |   |   |                |
| 23  | 22   | 44  | 275                                | 25                                 | 58                                      | 2                                       | 7                            | 19                       | 144                                | 6                                  | 28                                      | 2                                       | No             |
| 24  | 41   | 194                                       | 258                                | 42                                 | 56                                      | 5                                       |                              |                          |                                    |                                    |   |   | No             |
| 25  | 29   | 152                                       | 285                                | 28                                 | 59                                      | 4                                       | 9                            | No                       |                                    |                                    |   |   | No             |

\* Dog paralyzed in this leg from operation.

† Dog died from distemper.

namely, that the ablation elicited considerable delay in the establishment of the positive clove response. Even after the clove responses had become fairly regular and the differentiation tests were started there would be intervals in which these dogs would not respond to cloves. These periods account for the large number of clove and asafetida negatives in the differentiation tests.<sup>3</sup> It should be stated, however, that these periods of no response usually come early in the differentiation tests (rarely after a

<sup>3</sup> There were periods in which so many negative responses occurred in dog 22 for cloves and asafetida that the differential tests were not carried to completion. It should be noted however that the failures to respond to asafetida were proportionately not more numerous than for cloves.

hundred trials). On the other hand there were relatively fewer negative clove responses during the later differential tests than in unoperated animals; so that the clove reflex appears to have ultimately gained momentum and become more stereotyped as a result of the lesion.

It is in connection with the differential tests that the most striking results of frontal lobectomy are shown. Table 4, columns 3 to 6 and 9 to 12 reveal that these dogs were unable, during a 7 second interval, to acquire a negative response to asafetida in 300 clove and 60 asafetida trials for the first foreleg and in 150 clove and 30 asafetida trials for the second foreleg. In every instance the number of negative responses for cloves was proportionately as large and in some instances larger than for asafetida. In every dog the positive responses for asafetida came without hesitation and their general behavior was the same as during clove inhalation.

Dog 19 was presumably a very fit subject for these tests as shown by the fact that the first clove response after the lesion came with the third trial.<sup>4</sup> The 4th, 8th and 9th tests were also positive, but it was not until the 76th trial that the clove responses became sufficiently regular to warrant starting the asafetida tests. In the differential tests the ratio of this dog's positive to negative clove responses for the first foreleg was 303 to 30 and for asafetida 63 to 4; and for the second foreleg the clove ratios were 149 to 5 and asafetida 29 to 1. Kymograph records of the last differential test for the first foreleg show the 332nd clove, the 67th asafetida and the 333rd clove trials all positive in  $1\frac{1}{2}$ ,  $1\frac{1}{2}$  and 2 seconds respectively. Similar records of the last differential test for the second foreleg portray the last asafetida test grouped with two cloves and one xylol and all positive in 1 to 2 seconds. Several other purely olfactory vapors and vapors that were effective over both the olfactory and trigeminal nerves also elicited positive responses in this dog.

Columns 7 and 8 of table 4 disclose that four of these dogs had no more difficulty than normal dogs in being able to transfer the clove reflex from one foreleg to the other. However, dog 25 after 203 trials was unable to give a clove response with the second leg tested with any degree of regularity. It can be stated that the dog was not paralyzed in this leg and there was no more than normal degeneration in either pyramid.

Before completing the tests on the last dog of this series, work was started on another group to test the effect of frontal lobectomy on acquired olfactory conditioned reflexes, which quite unexpectedly disclosed that some of these normal dogs did not readily acquire a negative response to asafetida with the methods in vogue. To obtain this negative response with the required regularity in these dogs it was necessary to establish a

<sup>4</sup> With the increase in the number of dogs used in this investigation it has been found that an occasional dog required 50 to 60 trials to elicit the first positive response to cloves, while a few dogs acquired it in 2 to 4 trials.

new procedure which consisted of punishing a positive response to asafetida with a slap with a strap. Naturally these failures with normal dogs to establish a negative response to asafetida suggested the possibility that the data in table 4 might be from a chance run of dogs that lacked the normal ability to differentiate conditionally between cloves and asafetida with the methods used.

To test the validity of this possibility, dog 25 shown by table 4 to be unable to acquire the negative conditioned asafetida response after operating with the usual methods in 313 clove and 63 asafetida trials, was given the following additional tests and introducing punishment by a whip if asafetida evoked a positive response within 7 seconds. As a result of 5 series of differential tests involving 125 clove and 48 asafetida trials, 122 clove records were positive and 3 were negative, while 47 asafetida records were positive and one was negative. For the most part the usual routine of giving 5 cloves to one asafetida was maintained, but in a number of instances 5 clove trials were followed by 2 to 5 of asafetida. In this particular dog punishment by whip for an asafetida response had little or no inhibitory effect on the following test for asafetida or cloves. A kymographic record of the 48th asafetida test not only portrays a positive response in  $1\frac{1}{2}$  seconds, but this response is preceded and followed by similar positives for cloves.

But for the fact that a considerable amount of corroborative data will be introduced in a subsequent group of dogs in which conditioned differential tests were carried on both before and after frontal lobectomy, additional punishment tests as described for dog 25 would have been obtained for this group of dogs.

As for the actual damage from the extirpations—it is represented by the shaded area (fig. 1, *J*), which is from the brain of dog 19. Dog 22 was paralyzed in the right leg and the left pyramid was filled with degenerated sheaths. The other dogs of this group placed with both forelegs and did not exhibit motor symptoms. Also Marchi sections through the medulla revealed no more than the normal amount of degeneration in any pyramid.

*Conditioning preceded and followed ablation.* It is apparent from columns 1 and 2 of table 5 that we are dealing with perfectly normal dogs in so far as ability to acquire the positive foreleg response to cloves is concerned. As shown by column 3 of this table several of these dogs, notably 27 and 28, experienced considerable difficulty in acquiring the negative response to asafetida by the usual procedure and to hasten the process of differentiation positive responses to asafetida were punished by whipping. Before extirpation all 5 dogs were able to differentiate 75 per cent of the time or better between cloves and asafetida by using the usual sequence of 5 of cloves to 1 of asafetida. By correctly is meant that each negative asafetida response must be preceded and followed by positive clove responses.

After extirpation, table 5, columns 4 and 5 disclose that dog 30 did not respond to cloves until the 11th trial and the reflex did not become established until the 17th trial, which is practically a duplication of the number of tests used in learning the reflex before operating. On the other hand, the clove reflex was unaffected by the ablations in dogs 26 to 29.

As for conditioned differentiation between cloves and asafetida after frontal lobectomy it is clear from table 5, columns 6 to 10, that all 5 dogs of this group lost their ability to respond correctly in 7 seconds to cloves and to asafetida. The table shows that every dog responded positively to asafetida as frequently and in some instances more often than to cloves. This absence of differentiation took place not only when the original sequence of 5 cloves to 1 asafetida was maintained, but also in ratios of

TABLE 5  
*Effect of frontal lobectomy on acquired olfactory conditioned reflexes*

| DOG | BEFORE   |   |   | AFTER ABLATION               |                          |                                    |                                    |  |  |                |                              |                          |
|-----|--|---|---|------------------------------|--------------------------|------------------------------------|------------------------------------|--|--|----------------|------------------------------|--------------------------|
|     | First foreleg                                      |   |   | First foreleg                |                          |                                    |                                    |  |  |                | Second foreleg               |                          |
|     | First genuine clove response came with test number | Clove reflex established with test number | Negative response to asafetida established with test number | First genuine clove response | Clove reflex established | Differentiation tests              |                                    |  |  | Differentiates | First genuine clove response | Clove reflex established |
|     |  |   |   |                              |                          | Number of clove responses positive | Number of clove responses negative | Number of asafetida responses positive | Number of asafetida responses negative |                |                              |                          |
| 26  | 6  | 7   | 6   | 1                            | 7                        | 267                                | 8                                  | 53                                     | 2                                      | No             | 29                           | 100                      |
| 27  | 4  | 5   | 8   | 3                            | 3                        | 403                                | 25                                 | 108                                    | 5                                      | No             | 25                           | 51                       |
| 28  | 17   | 22  | 19  | 1                            | 2                        | 276                                | 24                                 | 71                                     | 6                                      | No             | 12                           | 22                       |
| 29  | 16   | 27  | 3   | 1                            | 2                        | 188                                | 12                                 | 50                                     | 3                                      | No             | 3                            | 4                        |
| 30  | 12   | 16  | 1   | 11                           | 17                       | 124                                | 26                                 | 20                                     | 5                                      | No             | 32                           | 41                       |

3 to 1, 5 to 5, 1 to 1, etc., using punishment by shock for clove negatives and whipping for positive responses to asafetida.

Dog 27, selected to typify the group, acquired the positive response to cloves with the 5th trial before frontal lobectomy. This dog, however, experienced some difficulty in learning not to respond to asafetida, but finally learned to differentiate perfectly between these two odors. After operating the clove reflex came regularly from the 3rd trial on. In the differential tests which followed this dog was given 428 clove and 113 asafetida trials, which resulted in 403 positives for cloves to 25 negatives and 108 positive responses for asafetida to 5 negatives. A typical differential record before ablation from dog 27, using the usual sequence of 5 to 1, shows a negative response to asafetida for 10 seconds preceded and

followed by clove responses in 1 and 2 seconds. A similar record after extirpation, using the 5 to 1 sequence reveals a positive response to asafetida within 2 seconds preceded and followed by clove responses in 2 and 4 seconds. The slight delay in the clove response following the asafetida positive may be due to inhibition from whipping or from a delayed inhalation. As illustrative of a series of asafetida trials in dog 27 after frontal lobectomy, in which each positive response to asafetida was punished by whipping, a kymograph record portrays 4 asafetida tests in series, preceded and followed by clove tests, all positive in 1 to 3 seconds. If these tests had been made before operating, at least 3 of the asafetida records would have been negative.

Table 5 shows that after removal of the frontal lobes, dog 30 led the other dogs of this group in the number of failures to respond during the differential tests; the ratio of failures, however, was proportionately the same for either odor. Not infrequently the first test after a positive response for asafetida, punished by whipping, resulted in a negative response irrespective of whether the vapor was cloves or asafetida. One kymograph record portrays a positive clove response, a positive asafetida response punished by whipping and a negative response for cloves in the order stated.

After completing the differentiation tests summarized in table 5 an additional attempt was made to secure conditioned differentiation from another angle with dogs 29 and 30. The new procedure was to develop, if possible, the negative response to asafetida perfectly by giving only asafetida tests and punishing all positive responses with whipping. If this method succeeded differentiation would be attempted as before. It should be emphasized that these two dogs had very different temperaments. Dog 29 was a very active high spirited spitz which seldom failed to give positive responses to both cloves and asafetida in the differentiation tests after frontal lobectomy. Dog 30, part bull, on the other hand would sometimes respond negatively to both cloves and asafetida and in about the same ratio.

As a result of 200 asafetida tests with the excitable spitz (no. 29), 178 were positive and 22 were negative. As for distribution of these negatives 4 came during the first series of 25 trials, none appeared in the last 3 series of 25 trials, and 7 and 8 respectively came during the 2nd and 4th series of tests. This greater number of negative responses can be explained by the fact that during these tests the anticipatory responses were more numerous during the intervals between trials and were punished by whipping. It should be noted that not more than 2 negatives were recorded successively and these repetitions occurred only 3 times widely separated. Since these tests were made 3 months after removing the frontal lobes and no progress was made in acquiring the negative response

for asafetida it seemed useless to introduce any additional differential tests for this dog.

Bull 30, however experienced no difficulty in acquiring a negative response to asafetida when this odor was used alone. A negative response came with the 2nd trial and became regular with the 4th on. The differential tests which followed revealed no improvement in conditioned differentiation over the original procedure. The first 5 clove tests were naturally negative due to inhibition carried over from the previous series of asafetida tests which yielded negative responses. The 6th and 7th clove tests were positive, the first asafetida was negative as was also the 8th clove after which a series of either cloves or asafetida could be run in which the responses would usually be correct, but as soon as these odors were interchanged the responses were wrong and there was no sign of differentiation. For example, a series of records shows the 19th clove test positive, the 20th negative, an asafetida positive and the 21st clove negative.

As for ability to transfer the conditioned clove reflex from one foreleg to the other after frontal lobectomy, table 5, columns 11 and 12 indicate that dogs 26, 27, and 30 experienced more than ordinary difficulty in transference of the reflex, while dogs 28 and 29, especially the latter, were not hampered in any way by the ablation.

All of the dogs with deleted frontal lobes were able to qualify perfectly in the differential food and no food test, that is, they were able to select and open when blindfolded a paper package containing meat from 3 paper packets of like size and texture. They all gave normal taste responses to solutions of sugar, salt, quinine and weak acetic acid.

The extent of the lesion in this group of dogs is shown by the shaded area (fig. 1, *J*) and Marchi sections through the medulla reveal little if any more than normal degeneration in the pyramids.

**DISCUSSION.** To help clarify the complicated anatomical relationships of the central olfactory system figure 2 is introduced. It is somewhat diagrammatic and the relationship of the pyriform-amygdaloid complex is based largely on Johnston's descriptions.

The question of localized association or correlation areas in the cerebrum is apparently as mooted as in the last century when defended by Hitzig, Ferrier, Flechsig, Horsley and Schäfer and opposed by Flourens, Munk, Goltz and Loeb. According to Frolov, Pavlov late in life regarded the cerebral cortex to be composed of two systems. 1. A region represented by a series of analyzers—sensory, motor, etc., concerned with conditioned or temporary reflexes. 2. A superstructure concerned with synthesis, generalization and abstraction located primarily in the frontal lobes.

As to the effect of decortication on smell, Dusser de Barenne states that highly organized smell reflexes persist in the absence of neocortex.

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Lebedinskaia and Rosenthal report the loss of smell but not taste after decortication. Bard and Rioch noted a changed personality and stereotyped reflexes as a result of removing the neocortex and varying portions of the paleocortex. One cat with a portion of the olfactory fibers and the

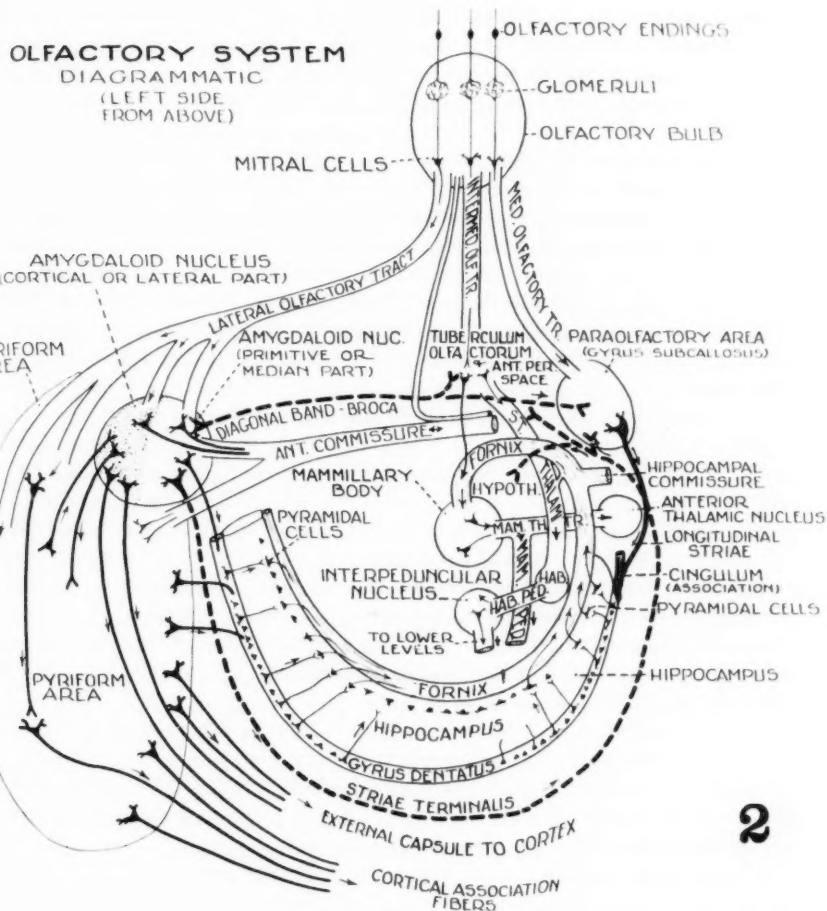


Fig. 2

basal olfactory area of one side more or less intact gave olfactory responses to food. Swann's work has been reported in an earlier paper.

In connection with the olfactory cortex, a correction should be made of a slip of citation in an earlier paper (p. 665) to read that Munk, Ferrier

and Luciani claim that a lateral lesion of the ventral portion of the temporal (not frontal) lobes abolished smell. Munk limited this area to the hippocampial gyrus, Ferrier to the hippocampial lobule (uncus, pyriform lobe and subiculum) and Luciani to the gyrus hippocampi and cornu ammonis. Ossipow has doubted the physiological relationship of the hippocampus to taste, smell, sight and hearing but calls attention to a relationship to cutaneous and muscle sense. Zavadsky, Henschen and Elsberg's papers have been reviewed previously. Johnston has concluded from comparative anatomical studies that the median portion of the amygdaloid nucleus and its hypothalamic connections are related to olfactory and taste correlation; while the lateral portion of the amygdaloid complex, a more recent nucleus phylogenetically, and the pyriform lobe is related to olfacto-somatic correlation. Hasami has recently recorded electrical potentials from the hippocampial (pyriform) lobe in the rabbit from inhalations of three odors. A few preliminary tests reported at the meeting of the American Physiological Society at Toronto suggest that the pyriform-amygdaloid complex (fig. 2 for connections) is an important area for supplying impulses that affect conditioned olfactory differentiation in dogs.

It is clear from the first part of this investigation that large extirpations of the parietal, occipital and temporal (exclusive of the hippocampi and pyriform areas) lobes or similar ablations of the hippocampi had little or no effect on *a*, acquired or acquiring a positive foreleg response to clove vapor; *b*, the transference of this reflex from one foreleg to the other; *c*, an established or establishing a negative response to asafetida and a conditioned olfactory differentiation which involved a decision in 7 seconds, whether to flex a foreleg for cloves and not flex it for asafetida; *d*, ability when blindfolded to find, select and open a paper package containing meat from 3 paper packages of like size and texture; *e*, taste responses to solutions of sugar, salt, quinine and acetic acid.

On account of their late evolutionary development the frontal lobes have long been regarded as important areas for cerebration, but not the only region concerned with association and correlation. Pavlov in the late years of his life regarded the frontal lobes as having highly complicated relationships between the organism and its environment.

Clinicians are somewhat divided as to the importance of this structure. McCarthy, Dandy, Spurling, Jefferson and others are not worried over the detrimental effects of its removal. Jefferson's patients who were mentally affected before operating were improved by operating and others who were rational before were the same after. He also emphasizes the importance of pressure and altered blood pressure from lesions of this area. Sachs calls attention to a peculiar indifference and lack of memory for recent events in his patients. One of Penfield and Evans' cases of unilateral frontal

lobectomy was of especial interest for two reasons: 1, because of the long intimate relationship between patient and physician, and 2, because the so-called defective planned administration as illustrated by the inability of the patient to prepare all of the dishes of a full course dinner was somewhat comparable to our bilateral results on dogs. Brickner has recently recorded somewhat similar effects following removal of one frontal lobe. The patient was said to be unable to synthesize separate thoughts, unable to make a correct report of the details involved in the transfer of stocks. The effects of Brickner's earlier case of removing both frontal lobes have been too widely quoted to need mentioning.

Animal experimentation has added support to the frontal lobes being important association areas. The results of Bianchi and Franz have been reviewed by Jacobsen. Fulton and Jacobsen produced hysteria in a frontal lobectomized monkey by introducing problems beyond his ability to solve. Jacobsen and Finan have attributed *inability to respond correctly to delayed response* as an effect of bilateral extirpation of the frontal lobes.

It is apparent from this study that all dogs with deleted frontal lobes yielded the first foreleg response to cloves as rapidly as an unoperated dog, but to acquire this reflex perfectly ordinarily required many more trials. As for the effect of frontal lobectomy on the acquired clove reflex, 2 dogs showed a slight delay and 3 showed no delay in the appearance of the reflex. As for the ability to transfer the clove reflex from one foreleg to the other all were able to transfer, but some required more than the normal number of times to accomplish it.

The pronounced effect of frontal lobectomy came with the differential tests in which none of these dogs, irrespective of whether they had learned to differentiate before the operation or not, were able in 7 seconds to respond correctly to cloves or asafetida. It was immaterial in these tests whether the original sequence of 5 cloves to 1 asafetida was maintained or whether a 3 to 1, 5 to 5 or 1 to 1 were used. Also the results were identical whether a negative clove was punished by shock and a positive asafetida was whipped or whether only the clove errors were punished.

Attention should be directed to the fact that differences of response varied with differences in temperament. This is well illustrated by some additional differential tests carried out on an excitable spitz and a part bull, both of which differentiated perfectly before frontal lobectomy and failed afterward. These differences occurred during an attempt to establish a negative response to asafetida by a series of tests in which each positive response to asafetida was punished by whipping. They resulted in the spitz being unable to hold back his responses to asafetida, while the bull after a few consecutive trials never responded to asafetida. After the bull had acquired the ability to respond negatively to asafetida, differentiation was started as before. It resulted in as hopeless mixup between

the responses to cloves and asafetida as occurred in the original differential tests.

Finally it is likewise clear that the dogs with ablated frontal lobes while unable to give correct foreleg responses to cloves and asafetida in 7 seconds were able to differentiate immediately by smell between a paper package containing meat and 3 paper packets of like size and texture. When blindfolded they would quickly find their way to a pan containing these packages, pick up and open the meat package and ignore the paper packages. The food differentiation test by smell is obviously a much more primitive type of synthesis. It should be recognized that the olfactory fibers enter the cerebrum much closer to the frontal lobes than the other analyzers and as has been suggested by Herrick and others their earlier phylogenetic appearance must have played a very important rôle in the development of the cerebrum as a correlating center. In addition olfactory sense is especially well-developed in dogs and one of their most important analyzers. Furthermore the frontal lobes of the dog while much better developed than some mammals are not comparable to man and it is well known that the function and importance of some brain areas have changed in evolutionary development.

#### SUMMARY AND CONCLUSIONS

The following tests were made after, or before and after, certain specific ablations with a view of ascertaining the relationships of these areas to olfaction. *a*, establishment of a foreleg conditioned reflex to clove vapor; *b*, transference of the clove reflex from one foreleg to the other; *c*, establishment of a negative conditioned response to asafetida and conditioned differentiation, which involved a decision in 7 seconds whether to respond positively to cloves or negatively to asafetida; *d*, food from no food discrimination by smell.

Extensive bilateral extirpations of the parietal, occipital and temporal (exclusive of the hippocampi and pyriform areas) lobes or removal of 90 to 100 per cent of the hippocampi (cornu ammonis) or unilateral frontal lobectomy had little, if any, effect on *a*, *b* and *c* irrespective of whether the reflexes had or had not been acquired.

Bilateral extirpation of the frontal lobes caused no delay in the first appearance of *a*, but produced considerable delay in the establishment of *a* as a reflex. All but one dog which had acquired *a* before the operation responded to *a* sufficiently early during the first series of tests (1st to 3rd trial) after operating to indicate that the reflex had been retained. Frontal lobectomy produced some delay in the establishment of *b* in some dogs and no delay in others. Procedure *c* was absolutely abolished in all of the frontal lobectomized dogs. Attention was called to some temperamental variations of *c* in the discussion.

None of the above cerebral extirpations affected *d* or interfered with taste responses to solutions of sugar, salt, quinine and acetic acid.

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#### REFERENCES

- ALLEN, W. F. This Journal **118**: 532, 1937.  
 Ibid. **121**: 657, 1938.  
 Ibid. **126**: 419, 1939.
- BARD AND RIOCH. Bull. Johns Hopkins Hosp. **60**: 73, 1937.
- BRICHTNER, R. M. The intellectual functions of the frontal lobes. New York City, 1936. Arch. Neurol. and Psychiat. **41**: 1166, 1939.
- DANDY, W. E. Bull. Johns Hopkins Hosp. **33**: 188, 1922.
- DUSSER DE BARENNE, J. G. Arch. Neurol and Psychiat. **30**: 884, 1933.
- FINAN, J. L. J. Neurophysiol. **2**: 208, 1939.
- FROLOV, Y. P. Pavlov and his school. New York, 1937.
- FULTON AND JACOBSEN. Advances Modern Biol. **4**: 113, 1935.
- HASAMI, B. Pflüger's Arch. **234**: 748, 1934.
- HERRICK, C. J. Proc. Nat. Acad. Sci. **19**: 7, 1933.
- JACOBSEN, C. F. Comp. Psychol. Monogr. **13**: 1, 1936.
- JEFFERSON, G. Brit. M. J. July, 199, 1937.
- JOHNSTON, J. B. J. Comp. Neurol. **35**: 337, 1923.
- LEBEDINSKAYA AND ROSENTHAL. Brain **58**: 412, 1935.
- MCCARTHY, J. Brain **5**: 559, 1883.
- OSSIPOW, V. P. Arch. f. Anat. u. Physiol. S.1: 1900.
- PENFIELD AND EVANS. Brain **58**: 115, 1935.
- SACHS, E. Brain **50**: 474, 1927.
- SPURLING, R. G. South. Med. J. **27**: 4, 1934.

## THE EFFECT OF ENDOCRINE EXTRACTS ON THE AMINO ACIDS IN THE BLOOD WITH INCIDENTAL FINDINGS ON THE BLOOD SUGAR AND UREA

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In the course of studies on protein metabolism on young children with the nephrotic syndrome Farr and MacFadyen (1) discovered that in such patients there existed a chronic plasma amino acid deficit. During acute episodes, which previously have been termed nephrotic crises by Farr (2), these observers noted a further precipitate drop in the plasma amino acid level which returned sharply to its precritical value concomitantly with recovery from the acute episode. Although many workers have confirmed the early work of Van Slyke and Meyer (3) on the constancy of the amino acid level in the blood under normal post-digestive conditions, and previous work has failed to reveal the above type of variability of the blood amino acid level under a variety of disease conditions, the new findings stimulated us to reexamine the question of factors affecting the level of plasma amino acid. Several workers have reported that it was possible to alter the level of the blood amino acids by certain endocrine products. However, the results of different observers have not been in complete agreement, and in an attempt to find some mechanism which might serve to explain, in part, the disturbances noted in our patients, we have reinvestigated some of the earlier aspects of the problem using newer techniques and extending it to include some of the more recently isolated endocrine substances.

**ANALYTICAL PROCEDURES.** Total plasma and cell amino nitrogen were estimated throughout by the gasometric ninhydrin method of MacFadyen and Van Slyke (4). Some analyses were also carried out in parallel using the Van Slyke (5) gasometric nitrous acid method. Blood sugar was determined by the method of Miller and Van Slyke (6). The hypobromite technique of Van Slyke and Kugel (7) was used for determining blood urea nitrogen. The nitrogen content of some of the materials used was determined by the Van Slyke (8) gasometric micro-Kjeldahl method.

**Materials used.** The growth-promoting factor of anterior beef pituitary and the metabolic factor (9) of the anterior swine pituitary were both

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generously furnished by Armour and Company. Test assays of the growth-factor were made by Armour and Company. The average gain in weight in 12 days by hypophysectomized rats was 10 grams when 0.02 cc. was given twice daily, and 19 grams when 0.05 cc. was given twice daily. The insulin, pitressin, adrenal cortical hormone (Eschatin), antuitrin S, testosterone propionate, thyroxin and epinephrin were all standard commercial products obtained on the open market. The plasma used for one series of injections was obtained from patients on the wards of this hospital and pooled. None of the patients had any ascertainable endocrine disorder. Analysis of some of the extracts used for their nitrogen content showed the following: anterior pituitary growth factor, total nitrogen 236 mgm. per cent; nonprotein nitrogen 9.5 mgm. per cent; antuitrin S total nitrogen 13.5 mgm. per cent; pitressin total nitrogen 5.4 mgm. per cent; eschatin total nitrogen 2.3 mgm. per cent.

**EXPERIMENTAL PROCEDURES.** All experiments were carried out on young bitches from two to six months of age. Each animal was fasted overnight before being used for an experiment. For the duration of each experiment the animal was kept in a fasting state but was given water to drink *ad lib.* At no time was anesthesia or sedation used on the animals. Blood for analysis was obtained from the jugular vein. Intravenous injections were also given into the jugular. In each experiment the zero hour blood was drawn just before the injection of any material. After injection blood was withdrawn at suitable intervals for analysis. Anterior pituitary growth-promoting extract was given in some instances intravenously and in some intraperitoneally. The dose used was 5 cc. per kilogram of body weight. Pitressin, anterior pituitary metabolic factor, antuitrin S, eschatin, testosterone propionate and thyroxin were given intravenously. Epinephrin and insulin were given subcutaneously. In each instance the dose was adjusted to the animal used to make experiments comparable on a per kilogram basis. The amounts used were chosen arbitrarily to be large enough to assure a response. Controls were carried out by fasting the dogs overnight, then withdrawing blood for analysis during the following day while keeping the dogs in a fasting state. The cells and plasma were separated by rapid centrifugation immediately after withdrawal. Amino acid analyses on the cells were always carried out within a short time of obtaining the sample as these values tended to rise if the cell suspensions were kept more than two hours at room temperature. The plasmas, on the other hand, were stable and there was no change in the values obtained even after several days in the icebox.

**RESULTS.** The results of representative experiments are shown in tables 1 to 6. They can be summarized by stating that anterior pituitary growth-promoting factor produced slight but consistent decreases in the plasma amino acids when given intraperitoneally with no change in cell

amino nitrogen (table 2). When, however, the material was injected intravenously a consistent and significant increase in the plasma amino acids was observed (table 3). Following intraperitoneal injections the animals seemed to experience considerable discomfort but no sharp reactions were encountered. If the anterior pituitary growth-factor was given intravenously it was uniformly followed by an immediate and severe

TABLE 1

*Plasma and cell amino nitrogen, blood sugar and blood urea nitrogen of dogs throughout a 24-hour fast and after pooled human sera intravenously*

| DOG NUMBER | SUBSTANCE INJECTED AND AMOUNT | TIME AFTER INJECTION | PLASMA AMINO NITROGEN | BLOOD SUGAR  | BLOOD UREA NITROGEN | CELL AMINO NITROGEN |
|------------|-------------------------------|----------------------|-----------------------|--------------|---------------------|---------------------|
|            |                               |                      | mgm./100 cc.          | mgm./100 cc. | mgm./100 cc.        | mgm./100 cc.        |
| 1          | Control                       | hours                |                       |              |                     |                     |
|            |                               | 0                    | 3.69                  | 79           | 9.9                 | 13.02               |
|            |                               | 6                    | 3.67                  | 74           | 8.4                 | 11.79               |
| 2          | Control                       | 24                   | 4.09                  | 86           | 14.0                | 12.87               |
|            |                               | 0                    | 4.39                  | 87           | 12.5                | 16.95               |
|            |                               | 6                    | 4.50                  | 94           | 11.8                | 16.38               |
| 1          | 8 cc. pooled sera             | 24                   | 4.47                  | 70           | 12.9                | 16.53               |
|            |                               | 0                    | 4.44                  |              | 12.9                |                     |
|            |                               | 3                    | 4.45                  |              | 14.0                |                     |
| 2          | 8 cc. pooled sera             | 6                    | 4.58                  |              | 13.4                |                     |
|            |                               | 0                    | 4.29                  |              | 18.4                |                     |
|            |                               | 3                    | 4.49                  |              | 18.6                |                     |
|            |                               | 6                    | 4.13                  |              | 15.8                |                     |

TABLE 2

*Effect of intraperitoneal injection of anterior pituitary growth factor on plasma and cell amino nitrogen, blood sugar and blood urea nitrogen*

| DOG NUMBER | DOSE | TIME AFTER INJECTION | PLASMA AMINO NITROGEN | BLOOD SUGAR  | BLOOD UREA NITROGEN | CELL AMINO NITROGEN |
|------------|------|----------------------|-----------------------|--------------|---------------------|---------------------|
|            |      |                      | mgm./100 cc.          | mgm./100 cc. | mgm./100 cc.        | mgm./100 cc.        |
| 1          | 5    | hours                |                       |              |                     |                     |
|            |      | 0                    | 3.93                  | 80           | 13.9                | 12.60               |
|            |      | 3                    | 4.02                  | 73           | 13.5                | 11.74               |
|            |      | 6                    | 4.55                  | 67           | 12.1                | 9.93                |
| 2          | 5    | 24                   | 5.24                  | 113          | 20.7                |                     |
|            |      | 0                    | 4.54                  | 85           | 11.6                | 13.23               |
|            |      | 3                    | 3.71                  | 84           | 11.6                | 12.75               |
|            |      | 6                    | 4.27                  | 77           | 9.0                 | 11.94               |
| 5          | 10   | 24                   | 4.23                  | 91           | 13.6                |                     |
|            |      | 0                    | 4.30                  | 87           | 7.2                 |                     |
|            |      | 1                    | 3.97                  | 87           | 6.6                 |                     |
|            |      | 2                    | 4.16                  | 90           | 6.8                 |                     |
| 1          | 15   | 3                    | 3.97                  | 90           | 10.3                |                     |
|            |      | 0                    | 4.00                  | 87           | 11.8                | 15.20               |
|            |      | 1                    | 3.71                  | 100          | 14.40               |                     |
|            |      | 2                    | 3.67                  | 94           | 12.1                | 16.20               |
| 2          | 15   | 3                    | 3.93                  | 83           | 12.6                | 14.05               |
|            |      | 6                    | 4.04                  | 83           |                     | 14.85               |
|            |      | 0                    | 4.21                  | 92           | 14.9                | 16.00               |
|            |      | 1                    | 3.62                  | 83           | 15.3                | 13.75               |
|            |      | 2                    | 3.76                  | 82           | 15.0                | 13.95               |
|            |      | 3                    | 4.33                  | 75           | 15.1                | 14.70               |
|            |      | 6                    | 3.92                  | 81           |                     | 15.30               |

reaction. This reaction often reached its maximum before injection could be completed. In many instances the dogs became comatose, an extreme degree of flaccidity and retching were always seen and frequently there was micturition. When returned to the cage the dogs would remain completely relaxed. After ten to fifteen minutes the animal would begin to look about and assume a more normal posture for a resting dog. In about twenty to twenty-five minutes the animals seemed to return to a normal

state and would get up on their feet and walk about the cage. However, about forty-five minutes had to elapse before the dogs would jump up in their cages to greet an observer. Insofar as we were able to judge, there was no further reaction and throughout the remainder of the day the animals seemed to be normal. These reactions were not dependent upon repeated injection of the material as they were seen after initial as well as subsequent injections with no change in the severity of the reactions during

TABLE 3

*Effect of intravenous injection of anterior pituitary growth factor upon plasma amino nitrogen, blood sugar and blood urea nitrogen*

| DOG NUMBER | DOSAGE  | TIME AFTER INJECTION | PLASMA AMINO NITROGEN | BLOOD SUGAR  | BLOOD UREA NITROGEN |
|------------|---|----------------------|-----------------------|--------------|---------------------|
|            |   | hours                | mgm./100 cc.          | mgm./100 cc. | mgm./100 cc.        |
| 3          | 11 cc.  | 0                    | 3.64                  | 68           | 9.5                 |
|            |   | 1                    | 3.87                  | 71           | 11.7                |
|            |   | 2                    | 3.80                  | 63           | 11.9                |
|            |   | 3                    | 5.00                  | 71           | 11.0                |
| 1          | 27 cc.  | 0                    | 3.92                  | 74           |                     |
|            |   | 1½                   | 3.99                  | 79           |                     |
|            |   | 3                    | 5.18                  | 71           |                     |
|            |   | 6                    | 6.23                  | 77           |                     |
| 1          | Desiccated powder in 9 cc. H <sub>2</sub> O = 27 cc. original | 0                    | 3.35                  | 83           |                     |
|            |   | 3                    | 4.54                  | 84           |                     |
|            |   | 5                    | 4.67                  | 87           |                     |
| 2          | Desiccated powder in 9 cc. H <sub>2</sub> O = 27 cc. original | 0                    | 3.83                  | 92           |                     |
|            |   | 3                    | 4.55                  | 87           |                     |
|            |   | 5                    | 4.42                  | 87           |                     |
| 2          | 17 cc. I.V. plus 15 cc. intraperitoneal                       | 0                    | 3.45                  | 78           |                     |
|            |   | 1½                   | 3.70                  | 76           |                     |
|            |   | 3                    | 4.33                  | 64           |                     |
|            |   | 6                    | 4.46                  | 73           |                     |

TABLE 4

*Effect of insulin subcutaneously, preceded by intraperitoneal and intravenous injections of anterior pituitary growth factor, on plasma amino nitrogen, blood sugar and blood urea nitrogen*

| DOG NUMBER | DOSAGE                                    | TIME AFTER INITIAL INJECTION | PLASMA AMINO NITROGEN | BLOOD SUGAR  | BLOOD UREA NITROGEN |
|------------|---|------------------------------|-----------------------|--------------|---------------------|
|            |   | hours                        | mgm./100 cc.          | mgm./100 cc. | mgm./100 cc.        |
| 1          | 20 cc. A.P.E. I.P.<br>10 units insulin    | 0                            | 4.07                  | 74           | 8.4                 |
|            |   | 1                            | 4.04                  | 77           | 7.3                 |
|            |   | 2                            | 3.57                  | 57           | 8.2                 |
|            |   | 3½                           | 2.85                  | 37           | 8.8                 |
| 2          | 20 cc. A.P.E. I.P.<br>10 units insulin    | 0                            | 3.84                  | 82           | 11.0                |
|            |   | 1                            | 4.01                  | 82           | 10.5                |
|            |   | 2                            | 2.93                  | 44           | 10.1                |
|            |   | 3½                           | 2.86                  | 33           |                     |
| 1          | 29 cc. A.P.E. I.V.<br><br>4 units insulin | 0                            | 2.90                  | 75           |                     |
|            |   | 0½                           | 3.52                  | 78           |                     |
|            |   | 1½                           | 3.48                  | 73           |                     |
|            |   | 3                            | 5.13                  | 87           |                     |
|            |   | 5                            | 5.46                  | 83           |                     |
| 2          | 29 cc. A.P.E. I.V.<br><br>4 units insulin | 6                            | 3.56                  | 46           |                     |
|            |   | 0                            | 3.08                  | 81           |                     |
|            |   | 0½                           | 3.65                  | 90           |                     |
|            |   | 1½                           | 3.90                  | 88           |                     |
|            |   | 5                            | 4.20                  | 95           |                     |
|            |   | 6                            | 2.92                  | 37           |                     |

the course of our experiments. Despite these severe seizures, no significant or consistent changes in either the blood sugar or the blood urea nitrogen were observed upon recovery from the reaction.

The administration of anterior pituitary metabolic factor was followed by no reaction though it was given intravenously (table 6). This material also caused an increase in the plasma amino acids with no detectable changes in the blood urea nitrogen or the blood sugar at the time of maximum alteration of the amino acid level.

TABLE 5

*Effect of insulin subcutaneously on plasma amino nitrogen, blood sugar, blood urea nitrogen and cell amino nitrogen*

| DOG<br>NUMBER | UNITS OF INSULIN          | TIME<br>AFTER<br>INJECTION | PLASMA<br>AMINO<br>NITROGEN | BLOOD<br>SUGAR   | BLOOD<br>UREA<br>NITROGEN | CELL<br>AMINO<br>NITROGEN |
|---------------|---------------------------|----------------------------|-----------------------------|------------------|---------------------------|---------------------------|
|               |                           | hours                      | mgm./<br>100 cc.            | mgm./<br>100 cc. | mgm./<br>100 cc.          | mgm./<br>100 cc.          |
| 1             | 5                         | 0                          | 3.23                        | 66               | 11.0                      | 16.47                     |
|               |                           | 1                          | 3.51                        | 71               | 10.8                      | 20.36                     |
|               |                           | 2                          | 3.90                        | 69               | 9.8                       | 19.68                     |
|               |                           | 4                          | 4.40                        | 71               | 9.1                       | 19.68                     |
| 2             | 5                         | 0                          | 4.43                        | 88               | 12.0                      | 19.14                     |
|               |                           | 1                          | 4.00                        | 38               | 12.3                      | 21.00                     |
|               |                           | 2                          | 3.24                        | 47               | 12.1                      | 17.91                     |
|               |                           | 4                          | 3.83                        | 68               | 13.3                      | 17.58                     |
| 1             | 10                        | 0                          | 3.98                        | 77               | 8.5                       | 19.44                     |
|               |                           | 1                          | 2.95                        | 35               | 7.9                       | 17.37                     |
|               |                           | 2                          | 2.46                        | 33               | 7.3                       | 18.45                     |
|               |                           | 4                          | 2.55                        | 25               | 6.0                       | 16.80                     |
| 2             | 5                         | 0                          | 3.93                        | 84               | 10.6                      | 18.48                     |
|               |                           | 1                          | 3.37                        | 31               | 10.6                      | 20.01                     |
|               | 7 cc. 50 per cent glucose | 2                          | 2.94                        | 27               |                           | 19.89                     |
|               |                           | 4                          | 2.69                        | 33               | 11.7                      | 18.54                     |
| 1             | 5                         | 0                          | 3.45                        | 77               | 5.1                       |                           |
|               |                           | 0 $\frac{1}{2}$            | 2.73                        | 40               | 4.4                       |                           |
|               |                           | 1                          | 2.85                        | 28               | 5.4                       |                           |
|               |                           | 2                          | 2.96                        | 27               | 5.0                       |                           |
| 2             | 2                         | 0                          | 3.83                        | 75               | 17.7                      |                           |
|               |                           | 0 $\frac{1}{2}$            | 3.36                        | 28               | 14.8                      |                           |
|               |                           | 1                          | 3.08                        | 32               | 17.1                      |                           |
|               |                           | 2                          | 2.62                        | 40               | 15.8                      |                           |
| 3             | 2                         | 0                          | 3.46                        | 89               | 11.7                      |                           |
|               |                           | 1                          | 2.58                        | 29               | 8.7                       |                           |
|               |                           | 2                          | 2.44                        | 37               | 11.5                      |                           |
| 5             | 2                         | 0                          | 3.85                        | 85               | 12.2                      |                           |
|               |                           | 1                          | 2.81                        | 33               | 11.8                      |                           |
|               |                           | 2                          | 2.82                        | 36               | 11.6                      |                           |

Pitressin, antuitrin S, eschatin, testosterone propionate, and thyroxin all caused an increase in the plasma amino acid level (table 6). No significant changes in the level of the blood sugar or blood urea nitrogen

TABLE 6

*Effect of intravenous administration of anterior pituitary metabolic factor, pitressin, eschatin, antuitrin S, testosterone propionate, epinephrin, and thyroxin on plasma amino nitrogen, blood sugar and blood urea nitrogen*

| DOG<br>NUM-<br>BER | SUBSTANCE USED          | QUANTITY MATERIAL INJECTED | TIME<br>AFTER<br>INJE-<br>CTION | PLASMA<br>AMINO<br>NITRO-<br>GEN | BLOOD<br>SUGAR   | BLOOD<br>UREA<br>NITRO-<br>GEN |
|--------------------|-------------------------|----------------------------|---------------------------------|----------------------------------|------------------|--------------------------------|
|                    |                         |                            | hours                           | mgm./<br>100 cc.                 | mgm./<br>100 cc. | mgm./<br>100 cc.               |
| 1                  | Pitressin               | 5 cc.                      | 0                               | 3.24                             | 85               | 13.1                           |
|                    |                         |                            | 3                               | 4.67                             | 85               | 19.4                           |
|                    |                         |                            | 5                               | 5.14                             | 83               | 13.7                           |
| 2                  | Pitressin               | 5 cc.                      | 0                               | 3.30                             | 82               | 18.8                           |
|                    |                         |                            | 3                               | 4.04                             | 92               | 22.4                           |
|                    |                         |                            | 5                               | 4.51                             | 78               | 21.2                           |
| 1                  | A.P.E. metabolic factor | 28 cc.                     | 0                               | 3.30                             | 102              |                                |
|                    |                         |                            | 1                               | 3.36                             | 88               |                                |
|                    |                         |                            | 2                               | 4.36                             | 78               |                                |
|                    |                         |                            | 3½                              | 5.06                             | 80               |                                |
|                    |                         |                            | 5                               | 4.75                             | 80               |                                |
| 2                  | A.P.E. metabolic factor | 26 cc.                     | 0                               | 3.22                             | 84               |                                |
|                    |                         |                            | 1                               | 3.01                             | 85               |                                |
|                    |                         |                            | 2                               | 3.44                             | 79               |                                |
|                    |                         |                            | 3½                              | 3.85                             | 82               |                                |
|                    |                         |                            | 5                               | 4.01                             | 81               |                                |
| 1                  | Eschatin                | 28 cc.                     | 0                               | 3.23                             | 94               | 9.6                            |
|                    |                         |                            | 3                               | 4.37                             | 91               | 10.4                           |
|                    |                         |                            | 5                               | 3.90                             | 85               | 9.2                            |
| 2                  | Eschatin                | 29 cc.                     | 0                               | 3.45                             | 97               | 14.8                           |
|                    |                         |                            | 3                               | 4.33                             | 81               | 17.7                           |
|                    |                         |                            | 5                               | 4.70                             | 83               | 17.8                           |
| 1                  | Antuitrin S             | 27 cc.                     | 0                               | 3.71                             | 67               | 14.2                           |
|                    |                         |                            | 3                               | 5.41                             | 57               | 17.1                           |
|                    |                         |                            | 5                               | 6.49                             | 60               | 18.3                           |
| 2                  | Antuitrin S             | 28 cc.                     | 0                               | 3.83                             | 77               | 22.4                           |
|                    |                         |                            | 3                               | 4.74                             | 67               | 22.4                           |
|                    |                         |                            | 5                               | 5.34                             | 71               | 22.0                           |
| 1                  | Testosterone propionate | 75 mgm. in 3 cc. oil       | 0                               | 3.38                             | 70               | 8.3                            |
|                    |                         |                            | 3                               | 4.65                             | 69               | 7.7                            |
|                    |                         |                            | 5                               | 4.25                             | 65               | 7.8                            |
| 2                  | Testosterone propionate | 75 mgm. in 3 cc. oil       | 0                               | 3.32                             | 80               | 15.4                           |
|                    |                         |                            | 3                               | 4.00                             | 76               | 14.1                           |
|                    |                         |                            | 5                               | 3.85                             | 81               | 14.9                           |

TABLE 6—*Concluded*

| DOG<br>NUM-<br>BER | SUBSTANCE USED | QUANTITY MATERIAL INJECTED | TIME<br>AFTER<br>INJEC-<br>TION | PLASMA<br>AMINO<br>NITRO-<br>GEN | BLOOD<br>SUGAR   | BLOOD<br>UREA<br>NITRO-<br>GEN |
|--------------------|----------------|----------------------------|---------------------------------|----------------------------------|------------------|--------------------------------|
|                    |                |                            | hours                           | mgm./<br>100 cc.                 | mgm./<br>100 cc. | mgm./<br>100 cc.               |
| 1                  | Epinephrin     | 2 cc.                      | 0                               | 4.93                             | 59               | 11.3                           |
|                    |                |                            | 1                               | 3.31                             | 114              | 9.4                            |
|                    |                |                            | 2                               | 3.69                             | 124              | 11.5                           |
|                    |                |                            | 3                               | 3.37                             | 86               | 12.9                           |
| 2                  | Epinephrin     | 2 cc.                      | 0                               | 4.15                             | 58               | 17.9                           |
|                    |                |                            | 1                               | 3.53                             | 112              | 16.0                           |
|                    |                |                            | 2                               | 3.72                             | 111              | 16.2                           |
|                    |                |                            | 3                               | 3.81                             | 69               | 19.7                           |
| 1                  | Thyroxin       | 20 mgm.                    | 0                               | 3.28                             | 75               | 7.5                            |
|                    |                |                            | 3                               | 4.08                             | 82               | 8.0                            |
|                    |                |                            | 5                               | 4.26                             | 80               | 8.6                            |
| 2                  | Thyroxin       | 20 mgm.                    | 0                               | 3.53                             | 81               | 15.0                           |
|                    |                |                            | 3                               | 4.36                             | 75               | 18.0                           |
|                    |                |                            | 5                               | 4.58                             | 82               | 21.0                           |

were observed with any of these substances. None of these products caused any reaction following intravenous administration.

Insulin uniformly produced a lowering of the blood sugar and the plasma amino acids (table 5), with the exception of the first experiment on dog 1 when there was an increase in the plasma amino acids and no change in the blood sugar. Subsequently in this dog insulin produced the usual fall in amino acid and sugar. The extent of the drop was in general proportional to the dose of insulin used. The blood sugar fell rapidly. The amino acids in the plasma also fell but in several instances the rate of fall of the amino acids was slower than that of the blood sugar. Minimal values were reached by both sugar and plasma amino acid in a short time after administration following which there was no further drop but the level tended to remain low for some time and then gradually return to normal.

Changes in the cell amino nitrogen paralleling the plasma variations were not observed. Because of technical difficulties in preparing the cell suspensions our observations are rather limited. Two of four dogs that received intraperitoneal injections of anterior pituitary growth-factor showed decreases in cell amino nitrogen values. The remaining two animals showed no change (see table 2). After insulin, when the plasma amino acids decreased sharply, there was no definite trend established in the cell values (see table 5), and although there was some change in a few

of the values this was not greater than that observed in two control animals (table 1). This relative insensitivity of cell amino acid to factors affecting the level of the plasma amino acids was also noted by Farr and MacFadyen (1) in nephrotic children after feeding protein. Although the cell amino acids are free in the sense that they can be readily determined without preceding hydrolysis treatment, they do not appear to be readily diffusible or to be in any simple equilibrium with the plasma amino acids. One difficulty encountered in interpreting values for the cells is that glutathione reacts quantitatively with ninhydrin as it has a free terminal alpha-amino carboxyl group. Since this substance constitutes a considerable fraction of the total amino acids it creates difficulties in measuring and interpreting small changes when separate glutathione estimations are lacking. In a few experiments in which anterior pituitary extract was given and followed by insulin, the effect of the latter did not seem to be in any way affected by the preceding treatment with anterior pituitary extract whether given intraperitoneally or intravenously (table 4). Whatever the level of the blood amino acids at the time insulin was given there was the characteristic fall in the expected time. Changes in the blood sugar level were in accord with the insulin dosage used and the anterior pituitary extract seemed to have no effect in decreasing the insulin action on sugar. Again no effect on the level of blood urea nitrogen was noted at any time in this series of experiments.

When epinephrin was given subcutaneously the plasma amino acids decreased while the blood sugar increased. In this instance the blood urea nitrogen of both dogs showed a slight drop at the end of the first hour following injection, subsequently rising again to slightly above the original value three hours after administration.

**DISCUSSION.** Most investigators are agreed that insulin injections lower the blood amino nitrogen (10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). On the other hand a few workers were unable consistently to obtain decreases in blood amino nitrogen after insulin (21, 22), and one group of investigators obtained a rise in amino acids after giving insulin and glucose (23). Insulin administered to adrenalectomized rabbits had no effect on the blood amino acids, whereas adrenalin in these rabbits produced a decrease in the amino acid level (24).

In contrast, adrenalin has been reported to have no effect on blood amino nitrogen in intact animals (25) or as causing an increase (26). Thyroxin administration is said to cause an increased deamination in the blood (27).

Injection of the growth-promoting factor of anterior pituitary has been found to cause a drop in amino acids, non-protein nitrogen and urea of the blood (28, 29, 30) and an apparently increased nitrogen assimilation (29, 30). On the other hand an increase of blood non-protein nitrogen

and urea has been reported as following pituitrin and pitressin injections while pitocin caused a decrease (31).

Some of the variations in results of different investigators may have been due to the methods employed for determining the level of blood amino acid. Comparative observations on blood using different techniques, i.e., colorimetric (32), gasometric nitrous acid (5), formaldehyde titration (33), have shown the unreliability of the colorimetric methods (34). None of the methods previously employed has the specificity of the ninhydrin reaction which we have employed (4).

Although previously reported effect of anterior pituitary growth extract in causing a decrease of blood amino acids indicated that this might be the mode of specific action of the hormone, this interpretation appears unlikely in view of the opposite effects which we obtained by varying the mode of administration. Furthermore, the fact that a wide variety of endocrine products administered intravenously also produced similar changes in the plasma amino acids points to a lack of specificity of action. On the other hand, it seems unlikely that the results we have obtained are solely of a non-specific nature for we observed changes in the amino acids in different directions with different hormones. Whereas most of those used caused an increase in the level of plasma amino acids, insulin and epinephrin, with opposite effects on the blood sugar, both caused a decrease in the plasma amino acids. These observations might all be interpreted as indicating that the mechanism controlling the level of plasma amino acids is highly susceptible to a variety of hormones. What this mechanism might be, or even if such a mechanism exists, we are at present unable to state. No adequate explanation is at hand for the disparity of the results following intraperitoneal and intravenous administration of anterior pituitary growth extract. A great deal more investigation is necessary to clarify adequately the metabolic changes observed before any satisfactory explanation can be put forth as to the possible mode of action of these substances on intermediary nitrogen metabolism.

#### CONCLUSIONS

1. The intravenous administration to dogs of the growth and metabolic factors of anterior pituitary extract, pitressin, antuitrin S, adrenal cortical hormone, testosterone propionate and thyroxin were each followed by marked increases in the concentration of amino acids in the blood plasma.
2. The intraperitoneal injection of growth factor of anterior pituitary extract produced a transient fall in plasma amino acids.
3. These findings were not accompanied by significant or consistent changes in blood sugar or urea.
4. Subcutaneous injections of insulin and epinephrin respectively produced sharp decreases in plasma amino acids with mutually opposite

effects on the blood sugar. This confirms the work of previous workers. Changes in blood urea were not significant.

5. Changes in the plasma amino acids after insulin were not accompanied by corresponding change in the cell amino acids.

## REFERENCES

- (1) FARR, L. E. AND D. A. MACFADYEN. *Am. J. Dis. Child.*, in press.
- (2) FARR, L. E. *Am. J. Dis. Child.* **58**: 939, 1939.
- (3) VAN SLYKE, D. D. AND G. M. MEYER. *J. Biol. Chem.* **16**: 231, 1913.
- (4) MACFADYEN, D. A. AND D. D. VAN SLYKE. In preparation.
- (5) VAN SLYKE, D. D. *J. Biol. Chem.* **83**: 425, 1929.
- (6) MILLER, B. F. AND D. D. VAN SLYKE. *J. Biol. Chem.* **114**: 583, 1936.
- (7) VAN SLYKE, D. D. AND V. H. KUGEL. *J. Biol. Chem.* **102**: 489, 1933.
- (8) VAN SLYKE, D. D. *J. Biol. Chem.* **71**: 235, 1927.
- (9) O'DONOVAN, D. K. *This Journal* **119**: 381, 1937.
- (10) WEICHMAN, E. *Ztschr. ges. Exper. Med.* **44**: 158, 1924.
- (11) FALKENHAUSEN, M. *Arch. Exper. Path. und Pharmacol.* **109**: 249, 1925.
- (12) TASHIRO, K. *Tohoku J. Exper. Med.* **7**: 268, 1926.
- (13) LUCK, J. M., G. MORRISON AND L. F. WILBUR. *J. Biol. Chem.* **77**: 151, 1928.
- (14) KIECH, V. C. AND J. M. LUCK. *J. Biol. Chem.* **78**: 257, 1928.
- (15) DANIELS, A. C. AND J. M. LUCK. *J. Biol. Chem.* **91**: 119, 1931.
- (16) LUCK, J. M. AND S. W. MORSE. *Biochem. J.* **27**: 1648, 1933.
- (17) POWERS, H. H. AND F. REIS. *J. Biol. Chem.* **101**: 523, 1933.
- (18) MARTENS, R. *Compt. rend. Soc. Biol.* **115**: 752, 1934.
- (19) KARR, S. E. AND V. H. KRIKORIAN. *J. Biol. Chem.* **81**: 421, 1929.
- (20) MIRSKY, I. *This Journal* **124**: 569, 1938.
- (21) GREENE, C. H., K. SANDIFORD AND H. ROSS. *J. Biol. Chem.* **58**: 845, 1924.
- (22) BISCHOFF, F. AND L. F. LONG. *J. Biol. Chem.* **84**: 629, 1929.
- (23) BOUCKAERT, J. P., P. P. DE NAYER AND W. CASSIMAN. *Compt. rend. Soc. Biol.* **117**: 257, 1934.
- (24) DAVIS, B. L., JR. AND W. VAN WINKLE, JR. *J. Biol. Chem.* **104**: 207, 1934.
- (25) OKADA, S. AND T. HAYASHI. *J. Biol. Chem.* **51**: 121, 1922.
- (26) BENHAMOU, E. AND R. GILLE. *Compt. rend. Soc. Biol.* **107**: 173, 1931.
- (27) MARTINI, E. *Boll. Soc. Ital. biol. sper.* **3**: 451, 1928.
- (28) TEEL, H. M. AND O. WATKINS. *This Journal* **89**: 662, 1929.
- (29) GAEBLER, O. H. *J. Exper. Med.* **57**: 349, 1933.
- (30) GAEBLER, O. H. AND W. H. PRICE. *J. Biol. Chem.* **121**: 497, 1937.
- (31) YOKOYAMA, E. *Sei-i-kar. Med. J.* **55**: 507, 1936.
- (32) FOLIN, O. *J. Biol. Chem.* **51**: 377, 1922.
- (33) SØRENSEN, S. P. L. *Compt. rend. trav. Lab. Carlsberg* **7**: 1, 1907.
- (34) VAN SLYKE, D. D. AND E. KIRK. *J. Biol. Chem.* **102**: 651, 1933.

## THE RELATIVE RÔLES OF THE EXTREMITIES IN THE DISSIPATION OF HEAT FROM THE HUMAN BODY UNDER VARIOUS ENVIRONMENTAL TEMPERATURES AND RELATIVE HUMIDITIES

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The temperature of the skin of any small area of the human body is the resultant of the heat brought to it largely by the blood and of the heat lost by the various dissipative processes. Hence, the amount of heat to be lost, in order to maintain normality of the internal temperature of the body, and the temperature and humidity of the environment into which this energy is to be dissipated, are the two chief factors which control the temperatures of various parts of the surface of the body if air movement is kept at a low value. Measurements of changes in skin temperatures of various areas of the body, under controlled environmental temperatures and relative humidities, have shown that the various portions of the body play relatively different rôles in the elimination of heat from the body. Within a range of atmospheric temperatures, frequently referred to as the zone of comfort and extending approximately from 23° to 30°C. (73° to 86°F.) in the case of the unclothed body, the regulation of the dissipation of heat is accomplished and controlled chiefly by the sympathetic nervous system through the production of vasoconstriction or vasodilatation of the peripheral vascular systems of the upper and lower extremities. While the exchange of heat between the body and its environment takes place over the entire surface of the body, the regulation of the loss of heat is maintained chiefly by vasomotor control of the peripheral blood vessels and consequent adjustments in the shifts of blood and changes in blood flow to the integument of the extremities.

In previous investigations on human subjects, studies have been made on the effects of various changes in the environmental temperature when the relative humidity, for the most part, was maintained at 25 or 40 per cent. The present investigation was carried out to determine the effects produced by changes in the relative humidity, in conjunction with changes in the environmental temperature, on the peripheral supply of blood to the extremities as evidenced by changes in skin temperature.

Measurements of changes in skin temperatures of various portions of the body under fairly well controlled environmental conditions, made by Pickering and Hess (1), Maddock and Coller (2) (3), Burton and Murlin (4) and Sheard, Williams and Horton (5), show that the extremities play a much more important rôle in the regulation of the dissipation of heat in the zone of vasomotor control than is exercised by the head and trunk. We have extended our investigations (6) regarding the relative rôles of the upper and lower extremities in the regulation and dissipation of heat from the body under temperatures ranging from 18° to 34°C. (64.5 to 93.2°F.) when these environmental temperatures were changed by steps of 1° to 2°C. while the relative humidity was maintained at 40 per cent. Table 1 gives representative data concerning the temperatures of the forehead, upper part of the leg, fingers and toes of two subjects systematically tested when in the basal state and under the specified dry-bulb temperatures. The basal metabolic rates for subjects A and B were 41.4 and 37.6 calories per square meter per hour.

Under atmospheric temperatures ranging from 18° to 22°C. (64.5 to 71.6°F.) the temperatures of the toes are, in general, at or near atmospheric temperature as the result of normal vasoconstriction, whereas, as the environmental temperature rises, increased vasodilatation occurs in the upper extremities, as is indicated by the rise in temperature of the fingers. When the fingers reach temperatures of 33° to 35°C. (91.4° to 95°F.), thus indicating approximately maximal vasodilatation, the temperatures of the toes generally exceed the environmental temperature. This ordinarily occurs under a room temperature of 25° to 26°C. (77° to 80°F.). Further increase of environmental temperature produces little if any change in the temperatures of the fingers; the temperatures of the toes, on the other hand, increase rapidly and reach a value comparable to the temperatures of the fingers under an environmental temperature of 28° to 29°C. (82.4° to 84.2°F.). Finally at higher environmental temperatures, 28° to 32°C. (82.4° to 89.6°F.), the temperatures of the fingers and toes closely approximate the temperatures of the forehead, thorax, legs and arms. At atmospheric temperatures exceeding about 31° to 32°C. (87.8° to 89.6°F.), maximal vasodilatation of peripheral blood vessels is maintained and the internal temperature of the body is kept approximately constant by changes in the secretion of sweat, and the chief regulation or control in the elimination of heat is by evaporation.

In previous investigations (5), it has been shown that the subjects should be in the basal metabolic state since, subsequent to the ingestion of food, there is an increase of skin temperatures of the fingers or toes respectively, dependent on the environmental temperature. Also, the subject should be in the supine position because of the effects of change of posture on the skin temperatures of the extremities (7). Furthermore,

the basal metabolic state must be taken into consideration in evaluating the relationships between skin temperatures of the extremities and the environmental temperature, since there is evidence to show that there is an approximately linear relationship (2), which may be of a dual character, however (8), existing between the average temperatures of the toes and the basal metabolic rates obtained under environmental conditions of 25°C. (77°F.) and 40 per cent relative humidity.

**PROCEDURE.** Data were obtained in psychometric rooms on two normal subjects with basal metabolic rates of 43.1 and 38.2 calories per square meter per hour. The subjects were fasted for fifteen hours previous to the tests. During the tests they wore light weight short pajamas and were in the supine position on a comfortable bed during the period from 9 a.m. to 5 p.m. The necessity of an adequate period for adjustment of the body to any given atmospheric temperature has been shown by Freeman and Linder (9). Hence, it is essential that the body remain under any given atmospheric conditions for an hour or more, or until fairly constant readings of the extremities are obtained. The temperatures of the plantar surfaces of the first and third toes of both feet, of the volar sides of the distal phalanges of the first and third toes of both feet, of the volar sides of the distal phalanges of the first and third fingers of the two hands, and of the forehead were measured by means of copper-constantan thermocouples (10). The changes in the environmental conditions were produced by means of controls which maintained the temperatures to  $\pm 1^\circ\text{F}$ . and the relative humidity to  $\pm 3$  per cent.

**RESULTS.** Our data show that, in subjects with normal circulation of the blood, approximately maximal vasodilatation of the peripheral vessels of the upper extremities exists in an environmental temperature of 25° to 26°C. (77° to 78.8°F.) and a relative humidity of 40 per cent. The high degree of vasodilatation of the peripheral blood vessels of the upper extremities is indicated by the fact that the temperatures of the fingers, which are the most sensitive indicators of the vasomotor regulation occurring in the upper extremities, are at the temperature of the forehead and thorax. On the other hand, the temperatures of the toes are at or but slightly above the room temperature (25°C.), thereby indicating considerable vasoconstriction in the lower extremities. In such conditions, therefore, the skin temperatures of the toes are the most sensitive or delicate indicators of the regulation of the loss of heat from the body as controlled chiefly by vasoconstriction of the peripheral vascular system of the lower extremities. Hence the finer or more precise control in the exchanges of heat between the body and its environment is accomplished by the lower extremities, as is most markedly shown by the skin temperatures of the toes. If, therefore, the dry bulb temperature of the environment is maintained constant, but the relative humidity is changed, it is possible to investigate the effects of changes of humidity on the dissipation

of heat from the body by means of measurements of the skin temperatures of the toes. Since the skin temperatures of the toes are sensitive indicators of the close regulatory control on the part of the sympathetic nervous system, it might be expected that, if observations were extended over an eight-hour period, there would be minor fluctuations in skin temperatures coupled with a slow decline of skin temperatures, particularly of the toes, on account of the slowly decreasing values of the metabolic rates (since no food was ingested during the twenty to twenty-four hour period of which any series of tests were a part) and the fatigue incident to the course of an eight-hour period of test. These gradual decreases in skin temperatures of the fingers or toes, which may indicate a change of  $2^{\circ}$  to  $3^{\circ}\text{C}$ .

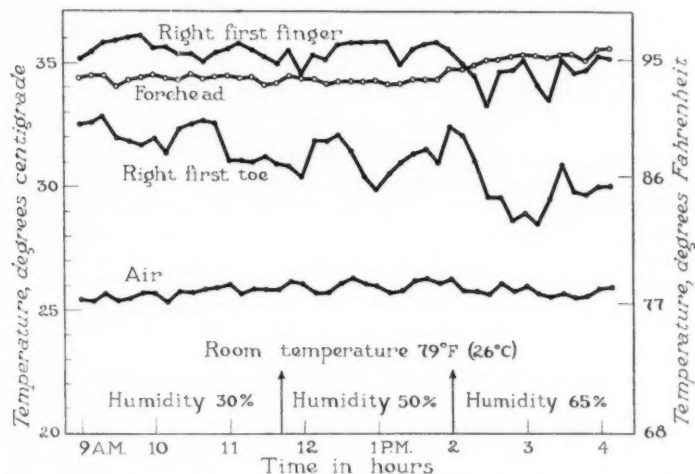


Fig. 1. Records of skin temperatures of the forehead, finger and toe of an individual placed in an environmental temperature of  $79^{\circ}\text{F}$ . ( $26^{\circ}\text{C}$ .) with 30, 50 and 65 per cent relative humidity respectively.

during the course of the day, are universally present in our investigations and are, therefore, exhibited in the curves of figures 1, 2 and 3.

If the environmental temperature is maintained at  $25^{\circ}$  to  $26^{\circ}\text{C}$ . and the relative humidity is changed from 30 to 50 to 65 per cent respectively, the curves of figure 1 and the data of tables 1 and 2 show that the skin temperatures of the extremities are nearly identical per se in all instances, indicating that the relative humidity has little effect on the loss of heat from the body. It is to be noted that the thermostatic control of the dissipation of heat was carried on by the lower extremities, since the upper extremities showed fairly constant and high vasodilatation as is evidenced by the temperatures of the fingers.

Since the principal rôle in the control of the dissipation of heat was

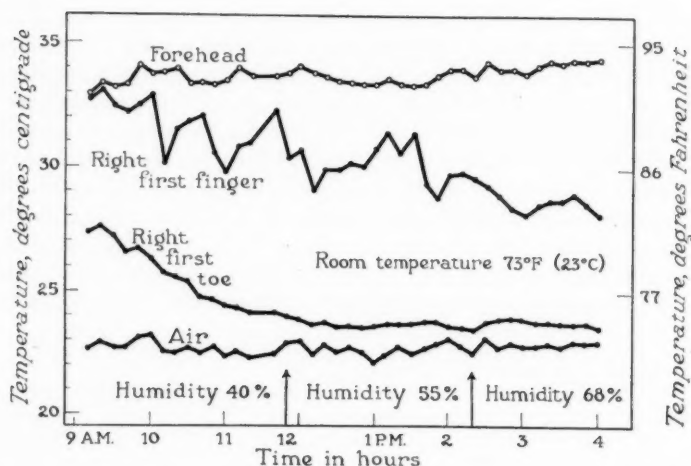


Fig. 2. Skin temperatures of the forehead, finger and toe of the same individual as in figure 1 under an atmospheric temperature of 73°F. (22.8°C.) when the relative humidity was 40, 55 and 68 per cent respectively, showing vasoconstriction of the upper and lower extremities dependent on the dry-bulb temperature of the environment.

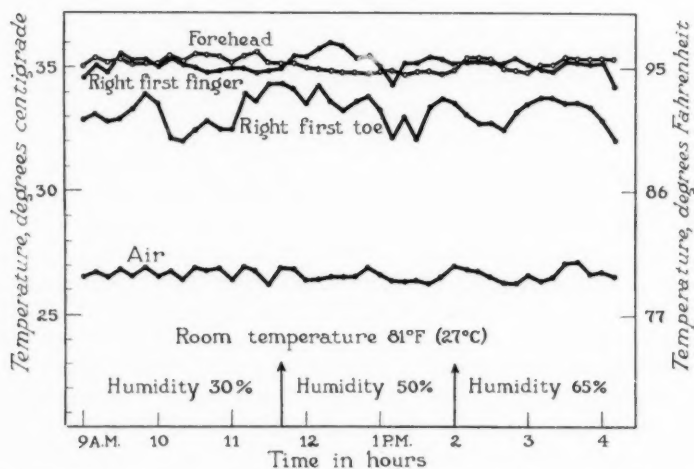


Fig. 3. Skin temperatures of the forehead, finger and toe of the same individual as in figures 1 and 2 under an atmospheric temperature of 81°F. (27.2°C.) with 30, 50 and 65 per cent relative humidity respectively, showing vasodilatation of the extremities which is not affected by changes in the relative humidity.

shown, in the foregoing observations, to reside in the lower extremities, the environmental temperature was decreased to 23°C. (73°F.). Vasoconstriction of the lower extremities exists at this environmental temperature and the finer rôle in the regulation of heat dissipation is exercised normally by the upper extremities. An atmospheric temperature of 23°C. was maintained constant (within  $\pm 0.5^\circ\text{C}.$ ) throughout the period of observation, while the relative humidity was changed from 40 to 55 and

TABLE 1

*Skin temperatures of selected areas of the body of normal subjects in the basal state under environmental temperatures maintained at various degrees.*

*(Relative humidity, 40 per cent)\**

| SUBJECT | ROOM TEMPERATURE |      | RANGE OF SKIN TEMPERATURE, °C. |                  |           |           |
|---------|------------------|------|--------------------------------|------------------|-----------|-----------|
|         | °F.              | °C.  | Forehead                       | Leg (above knee) | Fingers   | Toes      |
| A       | 67.3             | 19.6 | 32.3-32.9                      | 32.9-35.4        | 31.4-32.8 | 18.6-19.2 |
|         | 70.0             | 21.1 | 33.2-33.4                      | 31.7-32.8        | 32.2-34.3 | 20.7-22.9 |
|         | 72.9             | 22.8 | 32.7-33.8                      | 32.4-33.2        | 33.5-34.6 | 24.2-26.3 |
|         | 77.5             | 25.3 | 34.3-34.7                      | 33.4-34.0        | 33.9-34.4 | 27.6-28.9 |
|         | 79.0             | 26.0 | 34.6-34.9                      | 33.6-34.2        | 34.6-35.1 | 30.5-31.6 |
|         | 79.8             | 26.6 | 34.2-35.0                      | 32.9-33.9        | 34.5-36.2 | 32.7-33.8 |
|         | 84.0             | 29.0 | 34.6-35.2                      | 34.4-35.1        | 34.7-35.5 | 33.9-35.0 |
|         | 93.5             | 34.1 | 35.7-36.2                      | 34.9-35.4        | 34.5-36.4 | 35.2-35.7 |
| B       | 64.5             | 18.0 | 32.6-32.8                      | 29.5-31.9        | 23.8-27.9 | 17.1-18.2 |
|         | 69.0             | 20.5 | 34.0-34.7                      | 30.2-31.4        | 27.5-30.3 | 18.6-19.3 |
|         | 69.8             | 21.0 | 33.8-34.2                      | 30.9-32.6        | 31.3-34.2 | 19.9-20.6 |
|         | 72.5             | 22.5 | 34.0-34.2                      | 31.3-32.9        | 33.4-33.9 | 21.4-21.9 |
|         | 74.5             | 23.6 | 33.8-34.3                      | 32.4-33.2        | 32.6-34.0 | 21.8-23.2 |
|         | 76.0             | 24.5 | 33.6-34.5                      | 33.1-33.9        | 33.2-34.6 | 23.5-26.1 |
|         | 77.0             | 25.0 | 34.7-35.2                      | 32.7-34.0        | 35.2-35.8 | 27.9-29.7 |
|         | 78.8             | 26.0 | 34.1-34.9                      | 33.2-34.1        | 34.9-36.2 | 30.6-32.8 |
|         | 84.5             | 29.2 | 34.6-35.3                      | 33.6-34.3        | 35.3-36.0 | 32.9-33.7 |
|         | 90.4             | 32.4 | 35.4-36.1                      | 34.9-35.4        | 35.3-36.4 | 34.2-35.5 |

\* The range of skin temperatures for any of the specified areas occurred during a period of observation of four to six hours.

later to 68 per cent. Little effect was produced on the skin temperatures of the fingers and toes by such changes in the relative humidity (fig. 2).

When the environmental temperature was maintained at 27°C. (81°F.), but the relative humidity was changed from 30 to 50 and finally to 65 per cent, only minor changes in the dissipation of heat were produced under considerable change in humidity, as is shown by the measurements of the skin temperatures of the extremities (fig. 3). The skin temperatures of the fingers and toes indicated well defined vasodilatation, for the tempera-

tures of the toes were from 5° to 7°C., and of the fingers almost 10°C., respectively, above the atmospheric temperature.

Furthermore, it was of interest to determine whether changes in relative humidity produced changes in the skin temperatures of the fingers and/or toes when any specified air temperature within the range of comfort was maintained for two or more hours. A range of air temperatures from 21° to 27.5°C. (70° to 82°F.) was chosen because the loss of heat due to

TABLE 2

*Data regarding the skin temperatures of the fingers and toes of subjects placed under specified environmental temperatures and various relative humidities*

| ENVIRONMENTAL CONDITIONS |                                      | SUBJECT 1<br>AVERAGE TEMPERATURE |      | SUBJECT 2<br>AVERAGE TEMPERATURE |      |
|--------------------------|--------------------------------------|----------------------------------|------|----------------------------------|------|
| Dry-bulb temperature     | Relative humidity<br>(average value) | Fingers                          | Toes | Fingers                          | Toes |
| °C.                      | per cent                             | °C.                              | °C.  | °C.                              | °C.  |
| 21.1                     | 53                                   | 31.5                             | 22.1 | 30.2*                            | 21.3 |
| (70°F.)                  | 76                                   | 28.4                             | 23.3 | 26.5                             | 22.4 |
| 22.2                     | 40                                   | 28.5                             | 22.5 | 26.5                             | 22.1 |
| (72°F.)                  | 62                                   | 30.5                             | 25.6 | 31.0                             | 23.8 |
| 23.3                     | 40                                   | 34.8                             | 30.5 | 34.5                             | 29.5 |
| (74°F.)                  | 54                                   | 32.0                             | 25.4 | 31.7                             | 24.4 |
|                          | 68                                   | 28.0                             | 23.8 | 27.4                             | 23.6 |
| 24.5                     | 42                                   | 34.7                             | 29.5 | 33.5                             | 28.7 |
| (76°F.)                  | 54                                   | 34.0                             | 29.3 | 33.8                             | 28.8 |
|                          | 73                                   | 34.6                             | 29.5 | 34.3                             | 30.1 |
| 26.1                     | 38                                   | 35.1                             | 32.0 | 35.5                             | 32.5 |
| (79°F.)                  | 50                                   | 35.0                             | 32.6 | 35.1                             | 32.7 |
|                          | 64                                   | 35.7                             | 33.8 | 35.8                             | 34.6 |
| 27.8                     | 34                                   | 35.5                             | 33.9 | 35.6                             | 34.2 |
| (82°F.)                  | 53                                   | 35.7                             | 34.3 | 36.1                             | 34.0 |
|                          | 76                                   | 34.9                             | 33.8 | 35.1                             | 33.6 |

evaporation is nearly constant (20 to 25 per cent) and the subjects reported comfort except for some chilliness of the feet at the lowest environmental temperatures. The temperatures of the forehead ranged from 33.2° to 34.6°C. The data recorded in table 2 show that, within the range of normal variations of environmental ( $\pm 0.5^\circ\text{C}.$ ) and skin ( $\pm 0.2^\circ\text{C}.$ ) temperatures, there is little, if any, effect produced by considerable changes in relative humidity at any given atmospheric temperature within the range of 70° to 82°F: on the skin temperatures of the fingers and toes.

COMMENTS. In 1934 Burton (11) carried out physical experiments to determine how the humidity affects the loss of heat by convection and radiation from a hot body in still air. Burton's findings showed that the effect of change of humidity is negligible for humidities that are encountered in normal conditions (range of 35 to 86 per cent in his experiments). He concluded that, in estimating the measure of heat lost by radiation and convection by the excess temperature above that of the surrounding air, the dry-bulb temperature should be used rather than the wet-bulb reading or some combination of the two temperatures (12). The importance of the relationship between humidity and evaporation of sweat has been emphasized by DuBois and Hardy (13) (14).

In addition to the loss of heat by radiation, convection and conduction, there is the loss by evaporation (insensible loss) which remains practically constant and accounts for 20 to 25 per cent of the total loss of heat from the body in the zone of comfort or zone of vasomotor control and body cooling. In investigations of this character on normal human subjects, therefore, it is necessary to keep the environmental temperature below approximately 30°C. (86°F.), since maximal vasodilatation of the peripheral blood vessels is maintained and the internal temperature of the body is kept constant by the increased secretion of sweat which occurs above this temperature. Considerable evaporation of the increased production of sweat will occur at temperatures above 31°C. (87.8°F.) if the humidity is low. Fairly high relative humidities may cause small increments in the skin temperatures of various regions of the body, particularly of the fingers and toes, because the loss of heat by evaporation of water from the skin and lungs is much reduced under relatively high environmental temperatures and humidities.

In addition to our investigations, Winslow, Herrington and Gagge (15) have shown that, below an air temperature of 77°F. (25°C.) and for the clothed semireclining body, sweat secretion is minimal (about 20 to 25 per cent) and variations downward in the relative humidity of the atmosphere exert only a minor effect on evaporative heat loss. Under conditions of low air movement, and with a relative humidity of 30 to 35 per cent, the evaporative loss of heat is about 4 klm. calories per square meter per hour greater than with a relative humidity of 75 to 80 per cent. This is equivalent to changing the air temperatures by less than 1°F.

#### SUMMARY

These investigations show that relative humidity has little effect on the skin temperatures of the body, as indicated most sensitively by changes in the temperatures of the fingers and toes, when the body is subjected to fixed environmental temperatures ranging from approximately 23° to 28°C. (73° to 82°F.) with a fairly wide range (35 to 75 per cent) of relative

humidity. Any minor increases in skin temperature which may be produced by large increases in atmospheric humidity are overshadowed by the relatively large thermal changes in the extremities due to small changes in environmental temperature. Therefore, under the condition of these investigations the dissipation of heat from the body is dependent chiefly on the environmental temperature and is little influenced by relative humidity, when the person under test is in the basal metabolic state, lightly clothed and at rest in still air.

## REFERENCES

- (1) PICKERING, G. W. AND W. HESS. Clin. Sc. **1**: 213, 1933.
- (2) MADDOCK, W. G. AND F. A. COLLIER. This Journal **106**: 589, 1933.
- (3) COLLIER, F. A. AND W. G. MADDOCK. Ann. Surg. **100**: 983, 1934.
- (4) BURTON, A. C. AND J. R. MURLIN. J. Nutrition **9**: 281, 1935.
- (5) SHEARD, C., M. M. D. WILLIAMS AND B. T. HORTON. Tr. Am. Soc. Heat. and Vent. Eng. **43**: 115, 1937.
- (6) SHEARD, C., M. M. D. WILLIAMS, G. M. ROTH AND B. T. HORTON. Tr. Am. Soc. Heat and Vent. Eng. **45**: 1939 (in press).
- (7) ROTH, G. M., M. M. D. WILLIAMS AND C. SHEARD. This Journal **124**: 161, 1938.
- (8) SHEARD, C., B. T. HORTON AND M. M. D. WILLIAMS. Proc. Staff Meet., Mayo Clin. (in press).
- (9) FREEMAN, H. AND F. E. LINDER. Arch. Int. Med. **54**: 981, 1934.
- (10) SHEARD, C. Am. J. Clin. Path. **1**: 209, 1931.
- (11) BURTON, A. C. J. Nutrition **7**: 497, 1934.
- (12) HOUGHTEN, F. C. AND C. P. YAGLOGLOU. Tr. Am. Soc. Heat. and Vent. Eng. **29**: 361, 1923.
- (13) HARDY, J. D. AND E. F. DuBOIS. J. Nutrition **15**: 477, 1938.
- (14) DuBOIS, E. F. AND J. D. HARDY. (Abstr.) Science **87**: 430, 1938.
- (15) WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **124**: 692, 1938.





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